PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF COMMON SPICES AND THEIR MIX

by

Niseel Manandhar

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2018

Phytochemical and Antioxidant Activity of Common Spices and their Mix

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment of the requirements for the degree of B. Tech. in Food Technology

by

Niseel Manandhar

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

May, 2018

Tribhuvan University

Institution of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *"Phytochemical and Antioxidant Activity of Common Spices and their Mix"* presented by Niseel Manandhar has been accepted as the partial fulfillment of the requirements for Bachelor degree in Food Technology.

Dissertation committee

1. Chairperson ____________________________

(Mr. Basanta K. Rai, *Assoc. Prof.*)

2. External Examiner ____________________________

(Mr. Birendra Yadav, *Asst. Prof.)*

3. Supervisor ____________________________

(Mr. Man Kr. Tamang, *Teaching Asst.*)

4. Internal Examiner ____________________________

(Mr. Yadav K.C., *Asst. Prof.*)

May, 2018

Acknowledgement

I am deeply indebted to my guide Teaching Assistant Mr. Man Kr. Tamang Central Campus of Technology, Dharan for his excellent guidance, encouragement and inspirations throughout the endeavor.

I am eternally thankful to Prof. Dr.Dhan Bahadur Karki (Campus chief, Central Campus of Technology), Associate Professor Basanta Kumar Rai (HOD, Department of Food Technology) and Associate Professor Geeta Bhattarai (Chairperson, Central Department of Food Technology) for their generosity and co-operation in providing an opportunity and facilities to perform this work successfully.

Furthermore, I would like to thank my friends Samina Basnet, Anuradha Yadav, Rojina Subedi, Gajendra Bohora, Pankaj Dahal, Ajit Kumar Sah, Nabin Parajuli, Saroj Ghimire , Nikesh Niraula and my juniors Sanil Joshi, Roshan Lamichhane and Kumar Subedi for their co-operation to make this work successful. I am deeply indebted to all the teachers, all the technical and non-technical staffs of Central Campus of Technology and those who have directly and indirectly supported in my endeavor.

Above all, I am indebted to my parents without whose constant encouragements, inspirations and affection, I would never have reached this far, and this work would have never seen the light of the day.

Date of submission: May, 2018

Niseel Manandhar

Abstract

This study was aimed to assess the phytochemical and antioxidant activity of five common spices and to optimize the superior mixture of these spices. In the current study, the spices included cinnamon, cloves, timur, large cardamom and small cardamom which were bought from local markets of Dharan. These spices were steeped in 80% methanol for 12 h at room temperature. Then the extracts were filtered using Whatman No. 1 filter paper, transferred to brown bottle and stored at refrigerated temperature until analysis. The analysis was made for total phenol content, total flavonoid content, total tannin content and DPPH free radical scavenging activities.

From analysis it was found that the highest phenol content was found in cinnamon (158.14 ± 1.07 mg GAE/g) and the lowest was found in small cardamom (74.70 ± 3.20 mg GAE/g) among 5 spices. Similarly, highest flavonoid content was found in cloves (337.29 ± 10.59 mg QE/g) followed by cinnamon, large cardamom, timur and small cardamom. In the same way, highest tannin content was found in cinnamon. Cinnamon extracts had the highest antioxidant activity (68.74 ± 1.56 %) and small cardamom had the lowest among 5 spices. The Spiced mixture having cinnamon 0.6 parts and other spices 0.1 parts was found to be the optimized mixture which was significant at ($p < 0.05$).

Contents

Approval Letter iii

Acknowledgement iv

List of Figures xi

List of Plates xii

List of Abbreviations xiii

1. Introduction 1-3

1.1 General introduction 1

1.2 Statement of the problems 2

1.3 Objectives of the study 3

1.3.1 General objective 3

1.3.2 Specific objective 3

1.4 Significance of the study 3

1.5 Limitation of the study 3

2. Literature review 4-25

2.1 Spices 4

2.2.1 Classes of major phytochemicals, food sources and nutritional benefits ... 5

2.2.2 Phenolics / polyphenols 7

2.2.3 Significance of phenolic compounds 8

2.2.4 Flavonoids 9

2.2.5 Tannins 12

2.2.5.1 Activity of tannins 12

2.2.6 Phytochemical metabolism in human 13

2.3 Extraction of phytochemicals 14

2.3.1 Maceration 14

2.4 Antioxidants 14

2.4.1 Necessity of use of antioxidants 16

2.4.2 Types of antioxidant 16

2.4.2.1 Synthetic (artificial) antioxidant 16

2.4.2.2 Natural antioxidant 19

2.4.2.3 Synergestic antioxidants 20

2.4.3 Oxidative stress 20

2.4.4 Mechanism of action of antioxidants 21

2.4.5 Levels of antioxidant action 21

2.5 Some common spices 22

2.5.1 *Zanthoxylum aramatum* (Timur) 22

2.5.2 *Cinnamomum zeylanicum* (Cinnamon) 23

2.5.3 *Syzygium aromaticum* (Cloves) 23

2.5.4 *Amomum subulatum* (Large Cardamom) 24

2.5.5 *Elettaria cardamomum* (Small Cardamom) 25

3. Materials and methods 26-30

3.1 Materials 26

3.1.1 Spices 26

3.1.2 Chemicals and equipment 26

3.2 Methods 27

3.2.1 Preparation of plant extracts 27

3.2.2 Preliminary test of phytochemicals 27

3.2.2.1 Total phenol 27

3.2.2.2 Flavonoid 28

3.2.2.3 Tannin 28

3.2.2.4 Saponin 28

3.2.2.5 Alkaloid 28

3.2.3 Determination of total phenolic content 28

3.2.4 Determination of total flavonoid content 28

3.2.5 Determination of tannin content 29

3.2.6 Free radical scavanging capacity 29

3.2.7 Formulation of spiced mixture 29

4. Results and discussions 31-36

4.1 Phytochemical screening 31

4.2 Total phenol content 32

4.3 Total flavonoid content 32

4.4 Total tannin content 34

4.5 Total antioxidant activity 35

5. Conclusions and recommendations 37

5.1 Conclusions 37

5.2 Recommendations 37

6. Summary 38-39

References.. 40-51

Appendices 52-57

Color plates 58

List of Tables

Table No.	Title	Page no.
2.1	Phytochemicals, their uses and functions	6
2.2	Flavonoids and their food sources	10
2.3	Some important flavonoids and their functions	10
3.1	Chemical	26
3.2	Materials	27
4.1	Phytochemical screening	31
4.2	Total phenol content of methanolic extracts of spices	32
4.3	Total flavonoid content of methanolic extracts of spices	33
4.4	Total tannin content of methanolic extracts of spices	34
4.5	Total antioxidant activity of methanolic extracts of spices	35
4.6	Phytochemical and antioxidant activity of spiced mixture	36

List of Figures

Figure No.	Title	Page No.
2.1	Basic structure of flavonoid	11
2.2	Reaction of DPPH-free radical with an antioxidant	15
2.3	Chemical structure of TBHQ	17
2.4	Chemical structure of BHA	18
2.5	Chemical structure of Butylated Hydroxy Toluene	19

List of Plates

Plate No.	Title	Page No.
P1	Analysis of sample	58
P2	Measuring absorbance in spectrophotometer	58

List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of Variance
BHA	Butylated Hydroxy Anisole
BHT	Butylated Hydroxy Toulene
DCG	De-hydrodiconiferyl alcohol Glucosidase
DPPH	Diphenyl picryl hydrazyl
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Services
GAE	Gallic Acid Equivalent
GRAS	Generally Recognized As Safe
PG	Propyl Gallate
PHGPX	Phospholipid hydroperoxide glutathione peroxide
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
TBHQ	Tertiary Butlyated Hydroquinone
QE	Quercetin Equivalent

Part I

Introduction

1.1 General introduction

The role of plants in human health has extensively revealed due to the emergence of numerous advancements in the medicine and nutrition disciplines. The awareness of the benefits of plants in food as wealthy additives poses researchers to pursue for discovering the influence of such ingredients to the health of the human beings (Hinneburg *et al.*, 2006). Spices and herbs are well known food ingredients, which enhances the flavor and aroma of the supplemented foods. Botanically, spices are one class of the aromatic plants; they are mainly present in the tropical provinces (Kirk and Sawyer, 1991). Spices play an important role as flavoring agents in the diet and are used throughout the world. Spices refers to dried part of plant that contains volatiles oils or aromatic flavors such as, buds (cloves) , bark (cinnamon) , root (ginger) , berries (black pepper) ,seeds (cumin ,coriander) (Anon., 2014). Spices can be used as medicine because they are natural products easily absorbed by our bodies and generally do not have any adverse effects. Herbal remedies are an important source for the discovery of new antibiotics (Okpekon *et al.*, 2004).

Spices not only used for dietary purposes like aroma, color, taste, flavor and preservations of foods but also used as a medicine in traditional system of medicine. Spices have their own unique aroma and flavor which derived from phytochemical compounds (Prasad *et al.*, 2012). Most phytochemicals are secondary metabolites and have been reported to possess vast biological properties like anticancer, antioxidant, anti-inflammatory, antimicrobial and antidiabetic etc. Further, they may act as detoxifying, neuro-activator and immunity potentiating agents. In addition, some spices may decrease the platelets aggregation and involve in the modulation of hormone. The plant metabolites have a wide range of chemicals with different potency and exhibit more than one function. Around 150 secondary metabolites have been investigated and classified according to their physico-chemical characteristics and protective functions (Saxena *et al.*, 2001). Therefore, different secondary metabolites reported to be present in spices, such as alkaloids, steroids, tannins, phenolics , flavonoids, sterols, resins and fatty acids can be used as key chemical constituents for the standardization (Swadhini, 2011). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Ali and Alqurainy, 2006). Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues (Hayes, 2005)

Spices have been widely used as condiments for thousands of years because of their flavor, taste and color. Several spices have been used as medicinal plants in folk medicine for the treatment of various diseases because they contain many bioactive compounds and possess a lot of beneficial health effects. For example, some antioxidants from spices, such as curcumin (turmeric), eugenol (clove), and capsaicin (red pepper), were experimentally evidenced to control cellular oxidative stress due to their antioxidant properties and their capacity to block the production of reactive oxygen species and interfering with signal transduction pathways (Zheng *et al.*, 2016). Free radicals are naturally produced under aerobic conditions and are part of normal physiological processes, however, an excess of free radicals can damage all cellular macromolecules including proteins, carbohydrates, lipids, and nucleic acids (Bellomo, 1991).The free radicals initiate reactions such as the oxidation of DNA, which can ultimately cause mutations in the genetic material and possibly cancer. The oxidizing nature of free radical can result in enzymes inhibition or cause proteins to denature or degrade (Halliwell and Gutteridge, 1986). Antioxidants are substances that neutralize the harmful free radicals in our bodies. Antioxidants act as "free radical scavengers" and hence prevent or slow the damage done by these free radicals. Antioxidants function as reducing agents, ultimately removing free radical intermediates and preventing further oxidation (Lugemwa *et al.,* 2013).

1.2 Statement of the problems

Spices and herbs have been extensively studied in different countries because of the high antioxidant activity in certain spices and their beneficial effects on human health (Yashin *et al.*, 2017). As part of our diet, spices in addition to fruits and vegetables, could provide us with additional sources of natural antioxidants. Antioxidants from spices are a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic, diterpene, and vitamins. As reported by Kaefer and Milner (2008) in recent times measurement of dietary intake of spices is gaining much significance as various phytochemicals present in spices have been recognized to have health promoting benefits and preventive role in chronic diseases.

1.3 Objectives of the study

1.3.1 General objective

The general objective of this study is to assess the phytochemical and antioxidant activity of five locally available spices and their mix.

1.3.2 Specific objective

To fulfill the general objective, specific objective undertaken were as follows:

1. To perform phytochemical screening of common five spices.
2. To assess the phenol, flavonoids and tannin content of above spices.
3. To find out the antioxidant activity of extract of spices sample.
4. To find out the phytochemical and antioxidant activity of their mix.

1.4 Significance of the study

Spices, the predominant flavoring, coloring and aromatic agents in foods and beverages, are now gaining importance for their diversified uses. The nutritional, anti-oxidant, anti-microbial and medicinal properties of spices have far-reaching implications. In the present scenario, the anti-diabetic, anti-hyper cholesterolemic, anti-carcinogenic, anti-inflammatory effects of spices have paramount importance, as the key health issues of mankind nowadays are diabetes, cardio-vascular diseases, arthritis and cancer. Spices or their active principles could be used as possible ameliorative or preventive agents for these health disorders. This study will provide detailed information on phytochemical compositions and antioxidant of five spices for broader application in foods and other relevant areas.

1.5 Limitation of the study

1. Only methanolic extracts were studied. Extraction in other solvent like ethanol, petroleum ether, chloroform,etc. could not be performed.
2. Only one variety of each spices were used.

Part II

Literature review

2.1 Spices

Spices are dried aromatic plant products used to flavor foods and beverages. They include leaves (rosemary, sage), flowers and flower buds (clove), bulbs (garlic, onion), rhizomes (asafoetida), fruit (pepper, cardamom), and other parts of the plant. Frequently, blends of several spices are used. Their importance in ancient times is well documented not only for their flavoring, but also for their medicinal, preservative, and antioxidants properties (Shelef, 1984). Over the past two to three decades many beneficial effects of the common food spices on the health have been understood (Arques *et al.*, 2008). The United States Food and Drug Administration (FDA) defined spices as aromatic vegetative substances used for seasoning of food and from which no portion of any volatile oil or flavoring principles have been removed, and are free from artificial coloring matters, adulterants and impurities (Farrel, 1990).

Spices are ‘‘Generally Recognized As Safe’’ (GRAS) by the FDA, at least at concentrations commonly found in foods (Ugwuona, 2014).Spices are used as substances that increase the taste and variation of food. According to world health organization (WHO), more than 80% of the world’s population relies on traditional medicines for their primary health care needs (Jyothiprabha and Venkatachalam, 2016). The medicinal value of spices, which include leaves (coriander, mint), buds (clove), bulbs (garlic, onion),fruits (red chili, black pepper), stem(cinnamon), rhizomes (ginger) and other plant parts, have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods (Chitravadivu *et al.*, 2009). Spices contribute very minimal nutrients to menu because they are used a very small amount (Sunder, 2016). The bulk of the major components of spice materials consist of carbohydrate, protein and little minerals. Tannins, resins, pigments, volatile, essential and fixed oils which contribute to flavouring occur in traces and constitute only a small fraction of the dry matter (Cowan, 1999).

Spiced mixture is a combination of some common ground spices used widely in Indian cuisine as an antioxidant. The spices for spiced mixture are usually toasted at low temperature to bring out more flavor before the blending. It is usually added to a cuisine near the end of cooking. There's no single spiced mixture recipe found as the ingredients and differ according to region as well as cultural preferences. But for the most, spiced mixture includes coriander, cumin, cardamom, cloves, black pepper, cinnamon and nutmeg (Basu *et al.*, 2016).

2.2 Phytochemicals

Phytochemicals consist of a large group of naturally occurring non nutrient, biologically active compounds found in plants. As implied by the prefix “phyto” in the name, phytochemicals are basically produced only by plants. Phytochemicals act as natural defense system for the host plants and in addition provide colour, aroma and flavour. Plants use phytochemicals as natural protection from bacteria, fungi and viruses (Ramanthan *et al.*, 1989). More than 4000 of these compounds have been discovered and it is expected that scientists will yet discover many more phytochemicals in plant foods such as fruits, vegetables, legumes, cereals, herbs and spices (Rowland, 1999).

A number of phytochemical are known, some of which include: alkaloids, saponins, flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids. They do not only protect the plants but have enormous physiological activities in humans and animals. These include cancer prevention, antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more (Doss and Anand, 2012). Phytochemicals give hot pepper the burning sensation, onions and garlic the pungent flavour and tomatoes their red colour (Lesschaeve and Noble, 2005). Phytochemicals can have profound physiological effects, act as antioxidants, mimic body hormones and suppress development of diseases in the body (Hayes, 2005).

2.2.1 Classes of major phytochemicals, food sources and nutritional benefits

Phytochemicals are numerous and are found in all plant products, including fruits, vegetables, legumes, cereals, herbs and spices. No single plant material is naturally endowed with all the vital phytochemicals needed by human. Consequently, it is advised that a wide variety of plant materials, including fruits, vegetables, grains, herbs and spices be consumed in order to benefit maximally from the rich combination of phytochemicals. Most of the plant materials in human diets contain some important phytochemicals. Some good food sources of phytochemicals include cabbage, lettuce, tomatoes, Carrot, water melon, mangoes, pawpaw, grapes, oranges, apples, cashew apple and nut, mustard, pears, oats, sweet potatoes, whole wheat, beans, ginger, onions, red pepper spinach, sesame seed and garlic among others (Obeta, 2015).

According to Birt (2006), phytochemicals work in synergy and their effects when served together are stronger than the sum of the effects of parts served separately. The thousands of phytochemicals so far discovered are group-based. Table 2.1 shows some of these phytochemicals, their sources and biological functions.

Table 2.1 Phytochemicals, their uses and functions

Phytochemical classes	Phytochemicals	Sources	Potential nutritional benefits
Carotenoids	β-carotene, & carotene,lutein, lycopene	Tomato, pumpkin, Carrot, watermelon. Guava, dark yellow pink and red coloured vegetative fruits	Act as antioxidant Reduce level of cancer Producing enzymes Inhibit spread of cancer
Polyphenols	Tannins	Fruits, Legumes, Green vegetable, black tea	Exhibit anti-microbial and antioxidant activities Increase antioxidant activity Prevent proliferation of cancer Help speed excretion of carcinogen from the body
Flavonoid	Anthocyanine, Anthoxanthin	Beans, citrus fruits	Block access of carcinogen, prevents Malignant change in cells Prevents cancer
Saponins	Panaxadiol, panaxatriol	Potato, tomato, soybean, beans	Reduce glucose and glycerol uptake in the gut.

Terpenes	Mono-terpenes	Garlic, maize, ginger	Help detoxify carcinogens, inhibit spread of cancer
Isothiocyanates	Allylisothiocyanate, indoles,sulfuraphane	Cruciferous vegetables including cabbage, curli flower, broccoli	Suppress tumor growth, boost proliferation of cancer-fighting enzymes

Source: Birt (2006)

2.2.2 Phenolics / polyphenols

Polyphenols, which include more than 8,000 compounds, are a family of natural compounds widely distributed in the outer layers of plant as suspected from their protective function in the plants (Manach *et al.*, 2004). Polyphenols occur in all plant foods and contribute to the beneficial health effects of vegetables and fruit (Balch, 2000). They range from simple molecules such as phenolic acid to highly polymerized compounds, such as tannins. Phenolic acids account for about one third of the total intake of polyphenols in human diet. These compounds are capable of removing free radicals, chelating metal catalysts; activate antioxidant enzymes, reducing α- tocopherol radicals, and inhibiting oxidases (Oboh, 2006). As a result, they neutralize free radicals formed during normal physiological functioning of human body (Burns *et al.*, 2001). The antioxidant activity of phenols is due to their redox properties through which they act as hydrogen donors, singlet oxygen quenchers, reducing and metal chelating agents. There is a highly positive relationship between total phenols and antioxidant activity of many plant materials (Gulcin *et al.*, 2004).

Polyphenols are the most abundant antioxidants in the diet and are widespread constituents of fruits, vegetables, cereals, dry legumes, chocolate, and beverages, such as tea, coffee, or wine (Scalbert *et al.*, 2005). Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom (Shahidi, 1992). Structurally, they contain aromatic ring containing one or more hydroxyl groups (O' Connell and Fox, 2001). Based on the number of carbon atoms present in its structure, phenolics are categorized into five major groups

1. C_6 group: This group of phenol includes simple phenols and benzoquinones with six carbon atoms.

2. C_6C_n group: Phenolic acid and hydroxycinnamic acid derivatives are included in this group.
3. $C_6C_nC_6$ group: The largest group of phenolic compound includes flavonoids which have low molecular weight and are further of five types (flavones, flavonols, flavanols, flavanones and anthocyanins) on the basis of substitution pattern of carbon ring.
4. $(C_6C_n)_3$ group: This group consists of lignins and lignans.
5. Tannins: Tannins are high molecular weight phenols and classified into two main categories (hydrolysable and condensed tannins) (O' Connell and Fox, 2001)

The antioxidant capacity of polyphenols in any diet is much higher than the combined antioxidant effect of beta-carotene, vitamins A and E in the same diet (Gulcin *et al.*, 2004). The total intake of polyphenols in a person's diet could amount to 1g per day, whereas combined intakes of beta-carotene, vitamins C, and vitamin E from food most often is about 100 mg per day (King and Young, 1999). Important dietary sources of polyphenols include onions (flavonols), Cocoa (proanthocyanidins), tea, apples and red wine (flavonols and catechins), citrus fruit (flavanones), berries and cherries (anthocyanidins), and soybean (isoflavones). Polyphenol such as gallic acid and cathechin from natural products are used as standards when determining total phenol contents of plants and plant materials (Zahin *et al.*, 2009).

2.2.3 Significance of phenolic compounds

Phenolic compounds play various role in plants, few of which can be listed below

1. As antioxidant compounds: The main and most important role of phenol is their antioxidant property. They act as free radical scavengers which are formed due to high UV radiation.
2. As structural polymers: Lignin is the most important and widely distributed phenolic compounds which act as structural unit of plants.
3. As defensive compounds: Due to presence of tannins, plant develop an astringent taste. Tannins interact and precipitate with proteins which results in bitter taste of plants. Consequently, they act as feed deterrents in most of the cases.
4. As signal compounds: Many biochemical metabolic pathways have phenolic compounds as their signal molecules. For instance, in salicylic acid pathway, methyl

salicylate (phenolic compound) act as a signaling compound. Another phenolic signaling compound reported is de-hydrodiconiferyl alcohol glucosidase (DCG).

5. As pollinator attractors: Simple phenolic acids with low molecular weight are responsible for aroma and attractive coloration of flowers which attract pollinating agents.
6. As UV screen: The phenolics present in plant cuticle play an important role in screening the amount of UV radiations reaching earth through ozone layer.

2.2.4 Flavonoids

Flavonoids are the largest group of phenolic compounds and have a basic skeleton composed of three rings (C_6-C_3-C_6). They are classified into six major classes according to their substitution pattern in the B- and C-rings, which are flavan-3-ols, anthocyanins, flavones, isoflavones, flavanones and flavonols (J. Harborne and Baxter, 2000). The flavonoid polymers are also known as proanthocyanidins. Flavonoids occur as plant secondary metabolites that are involved in pigmentation, antioxidants, antimicrobials, antistressors, and UV irradiation protection (Vaya and Aviram, 2001). More than 4000 flavonoids have been described so far within the parts of plants normally consumed by humans and approximately 650 flavones and 1030 flavanols are known (Ghasemzadeh *et al.*, 2010).

Flavonoids are found in almost all plant based foods and beverages, but the levels vary, depending on the degree of ripeness of the fruits, variety and processing. Most flavonoids enhance the potency of vitamin C (ascorbic acid) and function as antioxidants (Su *et al.*, 2005). Antioxidant activity of flavonoids is believed to be due to their ability to act as free radical acceptor and to complex metal ions (Hertog *et al.*, 1992). They are biologically active against liver toxins, tumors, viruses and other microbes, allergies and inflammation (De *et al.*, 1999). Table 2.2 shows some flavonoid subclasses, specific examples of such subclass and their food sources while Table 2.3 shows specific flavonoid phytochemicals and their functions.

Table 2.2 Flavonoids and their food sources

Flavonoids	Subclasses	Food Sources
Flavonones	Hesperetin Eriodicatol Neringenin	Orange Lemon
Flavones	Lutelin Epigenin	Parsley, some cereals
Flavonols	Quercetin Kaempferol	Onion, tea Red wine, apple.
Flavanols	Catechin (monoma) Proanthocyanin (also called tannin)	Green tea
Anthocyanidine	Cyanidine	Berries, red wine, cherries
Isofavone	Genistein Daidzein Equol	Legumes, Soybean nuts, soy sauce.

Source: Rowland (1999)

Table 2.3 Some important flavonoids and their functions

S/N	Flavonoids	Functions
1	Hesperitin	Raises blood level of the “good cholesterol and lowers blood level of the “bad” cholesterol Prevents inflammation and relieves pains
2	Quercetin	Can prevent incidence of head and neck cancers Protects the lungs from harmful effects of pollutants and cigarette smoke
3	Tangeritin	Induces cell death in cancer cells (Leukemia) but promotes the life of normal healthy cells
4	Resveratol	May reduce the risks of heart diseases, stroke and blood clots.
5	Flavanols (Anthocyanins)	Act as potent antioxidant Helps to improve balanced coordination and short term memory in the elderly
6	Anthocyanins	Helps to prevent urinary tract infection.

Source: Hertog *et al.* (1992)

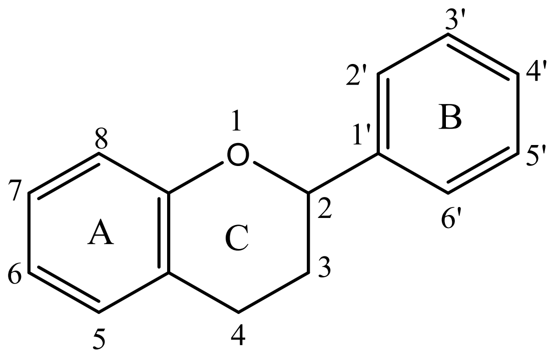


Fig. 2.1 Basic structure of flavonoid

Source: Hertog *et al.* (1992)

2.2.3.1 Biological activity of flavonoid

Flavonoids have gained recent attention because of their broad biological and pharmacological activities. They have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Ramos, 2008). The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities (Kelly *et al.*, 2002)

The β ring hydroxyl configuration is the most significant determinant of scavenging of ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Spevies) because it donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoids radical (Kumar and Pandey, 2013).

Mechanisms of antioxidant action may include

1. Suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation
2. Scavenging ROS
3. Regulation or protection of antioxidant defenses

Flavonoid action involves most of the mechanisms mentioned above. Some of the effects mediated by them may be the combined result of radical scavenging activity and the interaction with enzyme functions (Lewandowska *et al.*, 2015). Flavonoids inhibit the enzymes involved in ROS generation, that is, microsomal mono-oxygenase, glutathione s-transferase, mitochondrial succinoxidase, NADH oxidase, and so forth (Kumar and Pandey, 2013).

2.2.5 Tannins

Tannins are polyphenols sometimes called plant polyphenols although originally the name tannin was given to the plant extracts exhibiting astringency, without knowing their chemical structures (Haslam, 1989). The features distinguishing tannins from plant polyphenols of other types are basically the properties of the former: binding to proteins, basic compounds, pigments, large-molecular compounds and metallic ions, and also antioxidant activities, etc (Okadu and Ito, 2013). These are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. They form complexes with proteins, carbohydrates, gelatin and alkaloids. On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins (Saxena *et al.*, 2001).

1. Gallotannins are all those tannins in which galloyl units or their *meta*-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.
2. Ellagitannins are those tannins in which at least two galloyl units (C–C) are coupled to each other, and do not contain a glycosidically linked catechin unit.
3. Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of *C*-4 of one catechin with *C*-8 or *C*-6 of the next monomeric catechin.
4. Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit

2.2.5.1 Activity of tannins

Tannins have diverse effect on biological system since they are potential metal ion chelators, protein precipitating agents and biological antioxidants. Because of the varied biological roles that tannins can play and because of the enormous structural variation, it has become difficult to develop models that would allow an accurate prediction of their effects in any system (Skowyra, 2014).

The tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al.*, 2005).In the food industry tannins are used to clarify wine, beer, and fruit juices. Other industrial uses of tannins include textile dyes, as antioxidants in the fruit juice, beer, and wine industries, and as coagulants in rubber production (Gyamfi and Aniya, 2002). Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers. The search for new compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented (Palavy and Priscilla, 2006).

2.2.6 Phytochemical metabolism in human

Most phytochemicals found in foods exist in a variety of forms which influence their digestion and absorption. Most common ones are the polyphenols which exist as glycoside conjugates. Some glycosides must be digested to aglycones (unconjugated forms) before being absorbed. Some other forms of phytochemicals are thought to be absorbed in the intestines without intensive digestion. The absorption of most phytochemicals is thought to involve a carrier. Also, many glycosides are neither digested nor absorbed in the small intestines. Such phytochemicals not absorbed in the small intestine have been shown to undergo microbial degradation by colonic microflora (Ross and Kasum, 2002). The bacteria hydrolyse the glycosides, generating aglycones which may undergo further metabolism to form various aromatic compounds (Bradlaw *et al.,* 1999).

Once absorbed, most phytochemical metabolites get conjugated in the small intestine or in the Liver (Rhodes, 1996). Conjugation most often involves methylation, sulfating or glucunnidation. These conjugated metabolites are then bound to plasma proteins such as albumin and are transported through the blood to various parts of the body The amount of these conjugated metabolites in the plasma varies considerably with the type of polyphenol consumed, the food source, and the amount ingested (Kris-Etherton *et al.,* 2002). However, after consumption of specific polyphenols, little is known about the metabolism of the different polyphenols in the body, and also about what metabolites are present in the plasma (Briskin, 2000).

2.3 Extraction of phytochemicals

Extraction is the separation of medicinally active portions of plant using selective solvents through standard procedures (Handa *et al.*, 2008). There are different methods of extraction, the purpose of which is to separate the soluble plant metabolites, leaving behind the insoluble cellular residues (Angkawijaya *et al.*, 2014). The crude extract obtained through these methods contains complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids (Biesaga, 2011). Some of the crude extracts obtained may be ready for use as medicinal agents in the form of tinctures and as fluid extracts where as some need further processing. Several of the commonly used extraction methods are Maceration, infusion, decoction, soxhlet extraction, supercritical fluid extraction etc. (Azwanida, 2015).

2.3.1 Maceration

Maceration is a technique used in wine making and has been adopted and widely used in medicinal plants research. Maceration involves soaking plant materials (coarse or powdered) in a stoppered container with a polar solvent and allowed to stand at chilled temperature for a period of two to three days (Handa *et al.*, 2008). This process is intended to soften and break the plant cell wall to release the soluble phytochemicals. After two to three days, the mixture is pressed or strained by filtration. The choice of solvents will determine the type of compound extracted from the samples (Azwanida, 2015).

2.4 Antioxidants

Any substance which is capable of delaying, retarding or preventing the development of the rancidity or other flavors deterioration due to oxidation is called antioxidant (Coppen, 1983). Oxidation reactions are chemical reactions that involve the transfer of electrons from one substance to an oxidizing agent. Antioxidants can slow these reactions either by reacting with intermediates and halting the oxidation reaction directly, or by reacting with the oxidizing agent and preventing the oxidation reaction from occurring (Pokorny, 2007). Much is known about antioxidants because of their functional importance, interest in antioxidants is high to protect edible oils, their derived products and also when used in food products to provide baking and culinary characteristics and nutritional benefits (Chu and Chen, 2006). Antioxidants are substances that generally prevent, delay or retard the onset of rancidity in food products due to oxidation of the unsaturated fatty acids incorporated in food products. The use of antioxidants helps to extend the shelf life of a food, minimizes waste and nutritional losses, and extends the scope of use of various fats/oils (Bhattacharya, 2003).

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Chandra and Goyal, 2014).The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free DPPH radical with an odd electron gives absorbance (purple color) at 517nm. When the antioxidants in plant extract react with DPPH, it is reduced to DPPH-H and results in decolorization to yellow color with respect to the number of electrons captured. The color absorbance corresponds inversely to the radical scavenging activity of the sample extract. The scavenging of DPPH by radical scavengers can be summarized as

DPPH • + FE DPPH $\longrightarrow$ H + A• (1)

DPPH + A• DPPH $\longrightarrow$ A (2)

A• + A• A $\longrightarrow$ A (3)

Where FE is a scavenger of the extract and A• is a radical. The newly formed radical (A•) can mainly follow radical-radical interaction to render stable molecules, via radical disproportionate, collision of radicals with abstraction of an atom by one radical from another equations (Chandra and Goyal, 2014).

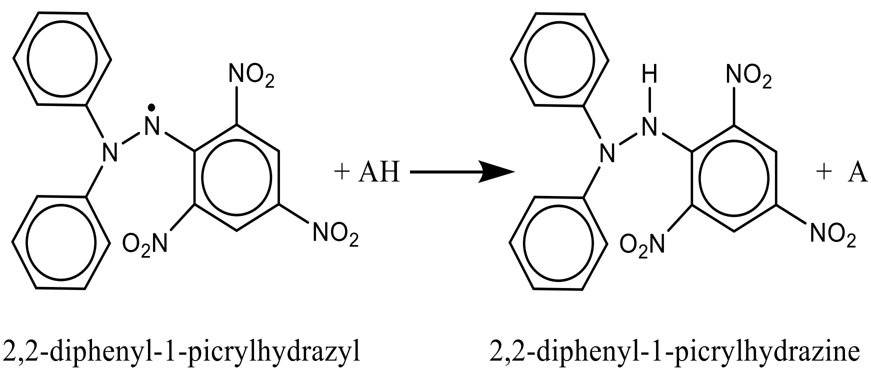


Fig. 2.2 Reaction of DPPH-free radical with an antioxidant

2.4.1 Necessity of use of antioxidants

Although techniques like vacuum packaging or packing under inert gas (to exclude oxygen) and refrigeration/freezing reduce the rate of autoxidation, they are not always applicable. Furthermore it is a fact that little oxygen is needed to initiate and maintain oxidative process and it is quite impossible or expensive to remove the least traces of air from a food product. Antioxidants are generally effective, easily applied and inexpensive. Other justification for need of an antioxidant use are- an antioxidant can extend the shelf life of a food, reduce wastage and nutritional losses (oil soluble vitamins) and more important it can widen the range of fats which can be used (Allen and Hamilton, 1983).

2.4.2 Types of antioxidant

Antioxidants are available in both natural and synthetic forms which are discussed separately below in the following section

2.4.2.1 Synthetic (artificial) antioxidant

Most of the synthetic antioxidants used are phenolic compounds among which Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA), Tert. Butylated Hydroquinone (TBHQ), Propyl Gallate (PG) are common in use. A quantitative tolerance limits for this synthetic antioxidant in Federal Regulations are limited not exceeds total content of 0.02% of fat or oils either alone or in combination (Bauernfeind and Cort, 1974). TBHQ, PG, BHA and BHT are the most commonly used antioxidants in food industries. In addition to being oxidizable, BHA and BHT are fat-soluble. Both molecules are incompatible with ferric salts. In addition to preserving foods, BHA and BHT are also used to preserve fats and oils in cosmetics and pharmaceuticals (FDA, 1992).

In recent years the possible toxicity of synthetic antioxidants has been investigated by several workers. Butylated Hydroxyanisole (BHA) has been banned. Most antioxidants are phenolic in structure and by the donation of a hydrogen atom to the acyl group of peroxy radical, they form relatively stable radicals and non-radical products. Japan following research by showed that the antioxidant promoted carcinogenesis in rats. Butylated Hydroxytoluene has also been implicated as a promoter of tumors (Imida *et al.*, 1983).

2.4.2.1.1 Tert-butylhydroquinone

Tert-Butylhydroquinone (TBHQ) is a synthetic food grade antioxidant that is used to stabilize foods, fats and vegetable oils against oxidative deterioration and thus extending their storage life. The structure of TBHQ is given in Fig. 2.4.

TBHQ is certified as safe for human consumption. In many major developing organizations like FDA (Food and Drug Administration), FSIS (Food Safety and Inspection Service) and others permit the use of TBHQ or combinations with BHA or BHT concentrations up to 0.02% by weight of the fat or oil content of the food (Anon., 2010).

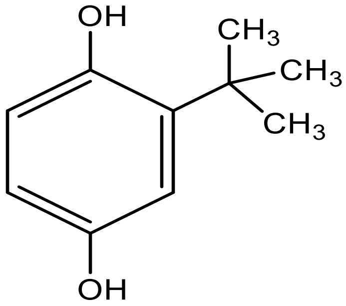


Fig. 2.3 Chemical structure of TBHQ

Source: Burr and Burr (1929)

2.4.2.1.2 Butylated hydroxyanisole (BHA)

Butylated hydroxyanisole (BHA) is a mixture of two isomeric organic compounds, 2-tertbutyl-4– hydroxyanisole and 3 – tert – butyl – 4 – hydroxyanisole. It is prepared from 4-methoxyphenol and isobutylene. It is a white or pale yellow solid (Crystal or Flake) with a faint aromatic odour (Burr and Burr, 1929).

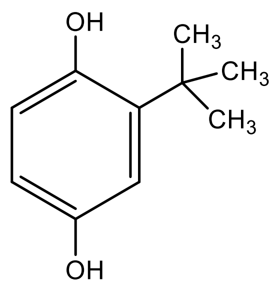


Fig. 2.4 Chemical structure of BHA

Source: Burr and Burr (1929)

2.4.2.1.3 Butylated hydroxy toluene (BHT)

Butlyated Hydroxyanisole is a synthetic antioxidant that is a commonly used fat soluble food preservative since 1947, with broad biological activities. It prevents spoilage by reacting with oxygen. It slows development of off-flavours, odours and colour changes caused by oxidation. It protects animal's aganist radiation and the acute toxicity of various xenobiotics and mutagens. Butylated hydroxyanisole (BHA) is a mixture of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole. It is prepared from 4-methoxyphenol and isobutylene. It is normally insoluble in water, but for commercial applications, it can be converted to a soluble form. BHT was first used as an antioxidant food additive in 1954. An antioxidant is a substance that prevents the oxidation of materials with which it occurs. BHT, therefore, prevents the spoilage of food to which it is added. BHT has grown to be very popular. Among food processors and is now used in a great range of products that include breakfast cereals, chewing gum, dried potato flakes, enriched rice, potato chips, candy, sausages, freeze-dried meats, and other foods containing fats and oils. BHT is sometimes used in conjunction with a related compound, Butylated hydroxyanisole (BHA) as a food additive.BHT does have other commercial uses, as in animal feeds and in the manufacture of synthetic rubber and plastics, where it also acts as an antioxidant (Bishov and Henic, 1977).

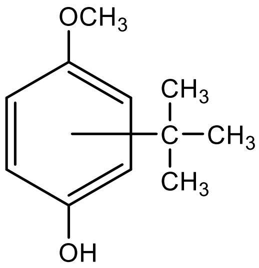


Fig 2.5: Chemical structure of butylated hydroxy toluene

Source: Burr and Burr (1929)

2.4.2.2 Natural antioxidant

Antioxidants are widely distributed in nature. Natural antioxidants impart no adverse effect in its long run of use; and do not have a quantitative tolerance limit in Federal Regulations. It imparts no adverse effect in its long run of use; natural antioxidants do not have a quantitative tolerance limit in federal regulations (Bauernfeind and Cort, 1974). Modern lipid science discovered that antioxidants are widely distributed in nature. Natural antioxidants seems to be more adequate for protection against oxidation and have many inherent qualities unsuppressed by the synthetic antioxidants (Burr and Burr, 1929).

Natural antioxidants can be used in a number of applications even where there is no choice for anything else, because of company policy or food legislations and public pressure group. There are some scientific evidences which alone is sufficient to support for using natural antioxidants. The antioxidants activity from natural sources has been demonstrated in spices (Chang *et al.*, 1977); vegetable extracts (Pratt and Watts, 1964) and plant protein and their hydrolysates (Bishov and Henic, 1977). None have achieved commercial importance. The tocopherols are an important group of natural antioxidants. Chipault (1862) and Aoes Jorgensen (1962) cover some its early work on tocopherol in vitro animal fat oxidation study showed that tocopherol have antioxidant activity at levels as low as (0.008%or 0.01%). Mixed with citric or ascorbyl palmate, tocopherol are efficient as BHT. In absence of oxygen, they are relatively heat and light stable (Bauernfeind and Cort, 1974).

The tocopherols are slightly viscous pale yellow liquids freely soluble in most organic solvents; insoluble in water. To retard the development of oxidative rancidity and tocopherols are used in foods as antioxidants. Tocopherols have a molecular wt. of 30-69 and boiling point 200-220 c (0.1 mm). In addition to food uses vitamin is used in food supplement and pharmaceutical dosage formulation. Tocopherols are readily oxidized and consequent protects the fat from oxidation (Meyer, 1987). Mode of action of natural antioxidants are not different from dose of similar synthetic phenols and polyphenols are proton donors which terminate free radical chins (Bishov and Henic, 1977).

2.4.2.3 Synergestic antioxidants

The preventive antioxidants which act by reducing the rate of chain initiation is called synergistic antioxidants although they have no effect as protectants when used along with fats (Lee, 1975).These compounds helps to increase (improve) the ability of the phenolic antioxidants to retard rancidity. (Furia, 1968) has presented an excellent review of the use of sequestrates (metal inactivators) in foods. Many components exhibit metal deactivating properties in edible triglyceride oils as evidenced by improvement in oxidative and/or flavor stability. Among these most important is citric acid (Dutton *et al.*, 1949).

All metal inactivating compounds have free hydroxyl for carboxyl groups that co-ordinate readily with metal forms salts readily (Cowan, 1999). Scalbert *et al.* (2005) proposed the metal inactivators, in effect complexes with peroxidant metal and hold them in a chelat or ring structure held winter co-ordination complexes, where the metal can no longer function as peroxidant.

2.4.3 Oxidative stress

The term is used to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable (Rock *et al.*, 1996). Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids (McCord, 2000). Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins, and excessive exercise. These injured tissues produce increased radical generating enzymes (e.g., xanthine oxidase, lipogenase, cyclooxygenase) activation of phagocytes, release of free iron, copper ions, or a disruption of the electron transport chains of oxidative phosphorylation, producing excess ROS. The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease (Rao *et al.*, 2006).

2.4.4 Mechanism of action of antioxidants

Two principle mechanisms of action have been proposed for antioxidants (Rice-Evans and Diplock, 1993). The first is a chain- breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation (Krinsky, 1992).

2.4.5 Levels of antioxidant action

The antioxidants acting in the defense systems act at different levels such as preventive, radical scavenging, repair and de novo, and the fourth line of defense, i.e., the adaptation (Lobo *et al.*, 2010).

The first line of defense is the preventive antioxidants, which suppress the formation of free radicals. Although the precise mechanism and site of radical formation in vivo are not well elucidated yet, the metal-induced decompositions of hydroperoxides and hydrogen peroxide must be one of the important sources. To suppress such reactions, some antioxidants reduce hydroperoxides and hydrogen peroxide beforehand to alcohols and water, respectively, without generation of free radicals and some proteins sequester metal ions (Lobo *et al.*, 2010).

Glutathione peroxidase, glutathione-s-transferase, phospholipid hydroperoxide glutathione peroxidase (PHGPX), and peroxidase are known to decompose lipid hydroperoxides to corresponding alcohols. PHGPX is unique in that it can reduce hydroperoxides of phospholipids integrated into biomembranes. Glutathione peroxidase and catalase reduce hydrogen peroxide to water (Lobo *et al.*, 2010).

The second line of defense is the antioxidants that scavenge the active radicals to suppress chain initiation and/or break the chain propagation reactions. Various endogenous radical-scavenging antioxidants are known: some are hydrophilic and others are lipophilic. Vitamin C, uric acid, bilirubin, albumin, and thiols are hydrophilic, radical-scavenging antioxidants, while vitamin E and ubiquinol are lipophilic radical-scavenging antioxidants. Vitamin E is accepted as the most potent radical-scavenging lipophilic antioxidant (Lobo *et al.*, 2010).

The third line of defense is the repair and de novo antioxidants. The proteolytic enzymes, proteinases, proteases, and peptidases, present in the cytosol and in the mitochondria of mammalian cells, recognize, degrade, and remove oxidatively modified proteins and prevent the accumulation of oxidized proteins (Lobo *et al.*, 2010)

The DNA repair systems also play an important role in the total defense system against oxidative damage. Various kinds of enzymes such as glycosylases and nucleases, which repair the damaged DNA, are known.There is another important function called adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site (Lobo *et al.*, 2010).

2.5 Some common spices

2.5.1 *Zanthoxylum aramatum* (Timur)

Zanthoxylum aramatum is a small xerophytic tree or shrubs. Leaflet blades usually with prickles. Leaves are compound, imparipinnate with 3-7 foliolate and pellucidpunctate. Petiole and rachis are winged. Leaflets are sessile, elliptical to ovate-lanceolate with cranate or entire margins. Flowers are brown axiliary, minute and polygamous. Calyx is 6-8 acute lobed, white petals are absent. Male flowers have 6-8 stamens with rudimentary ovary.Female flowewrs possess 1-3 carpels and 1-3 locular ovary. Fruit is drupe and small with red color, splitting into two when ripe. Seed are rounded and shining black (Barkatullah *et al.*, 2017).

The bark, fruits and seeds of *Z. armatum* are extensively used in indigeneous system of medicine as a carminative, stomachic and anthelmintic. The fruit and seeds are employed as an aromatic tonic in fever and dyspensia. An extract of the fruits is reported to be effective in expelling round worms. Because of their deodorant, disinfectant and antiseptic properties, the fruits are used in dental troubles, and their lotion for scabies (Singh and Singh, 2011). They are also used to ward off houseflies (Gaur, 1999). It is also used in India and China as snake bite remedy (Province, 1978). In Nepal, fruit decoction is used for abdominal pain (Rajbhandari, 2001). Berries are carminative, antispasmodic and used for rheumatism and skin diseases. Bark is used for cholera diabetes and asthma (Baral and Kurmi, 2006). Pickles from the fruits are useful for cold and cough, tonsillitis, headache fever, high altitude sickness, limbs numbness, diarrhoea and dysentery. Powdered dried fruits are taken with hot water to cure diarrhea, dysentery and stomach ache (Geweli and Awale, 2008).

2.5.2 *Cinnamomum zeylanicum* (Cinnamon)

Cinnamon (*Cinnamomum zeylanicum*, synonym *C. verum*) is a small evergreen tree, 10-15 meters (32.8-49.2 feet) tall, belonging to the family Lauraceae, native to Sri Lanka and South India. The flowers, which are arranged in panicles, have a greenish colour and have a distinct odour. The fruit is a purple one-centimeter berry containing a single seed (Filho *et al.*, 1999). Its flavour is due to an aromatic essential oil which makes up 0.5 to 1% of its composition (Jakhetia *et al.*, 2010).

In addition to being used as a spice and flavoring agent, cinnamon is also added to flavor chewing gums due to its mouth refreshing effects and ability to remove bad breath (Matan *et al.*, 2006). Cinnamon can also improve the health of the colon, thereby reducing the risk of colon cancer (Kim *et al.*, 206). Cinnamon is a coagulant and prevents bleeding (Subash Babu *et al.*, 2007). Cinnamon also increases the blood circulation in the uterus and advances tissue regeneration . This plant plays a vital role as a spice, but its essential oils and other constituents also have important activities, including antimicrobial (Crawford, 2009), antifungal (Roussel *et al.*, 2009), antioxidant (Suppapitiporn *et al.*, 2006), and antidiabetic. Cinnamon has been used as anti-inflammatory, antitermitic, nematicidal, mosquito larvicidal, insecticidal, antimycotic and anticancer agent (Kumari and Jain, 2015) . Cinnamon has also been traditionally used as tooth powder and to treat toothaches, dental problems, oral microbiota, and bad breath (Mang *et al.*, 2009).

2.5.3 *Syzygium aromaticum* (Cloves)

Syzygium aromaticum (S. *aromaticum*) (synonym: *Eugenia cariophylata*) commonly known as clove, is an median size tree (8-12 m) from the Mirtaceae family native from the Maluku islands in east Indonesia (Vavaiya *et al.* (2012). For centuries the trade of clove and the search of this valuable spice stimulated the economic development of this Asiatic region (Kamatou *et al.*, 2012).

Clove is a small evergreen tree with smooth gray bark and large, bright green, aromatic and lanceolate shaped leaves. The flowers grow in yellow to bright red clusters at the end of branches (Grieve, 1931). It is in the Myrtaceae family, with relatives ranging from guava to allspice to eucalyptus. The clove of commerce is the pink or reddish flower bud that turns dark brown when dried (Blumenthal *et al.*, 1998). The entire tree is highly aromatic and its Latin specific name aromaticum, refers to this intense aroma. The generic name Syzygium is based on the Greek word 'syzygos', that means 'paired or joined' and is in reference to the petals which are joined (Katzer, 2014). Clove bud is aromatic, a stimulant and carminative and is used for dyspepsia and gastric irritation (Adaramola and Onigbinde, 2016). The common name 'clove,' a derivative of the Latin 'clavus' meaning 'nail,' and refers to the shape of the clove (Mashkor (2015). Clove is native to the Maluku or Molucca Islands (often referred to as the Spice Islands, due, in part, to the abundance of clove) in Indonesia (Khalsa Singh and Tierra, 2008).

2.5.4 *Amomum subulatum* (Large Cardamom)

Large cardamom (*Amomum subulatum* Roxb.), a member of Zingiberaceae family under the order Scitaminae is a perennial soft-stemmed plant. The large cardamom plant is 1.5 to 2.5 m tall (Bisht *et al.*, 2011). It grows in the vicinity of mountain streams in swampy, cool, and humid areas in the shade of forest trees, of which nitrogenfixing trees are themore suitable shade trees. The Himalayan alder (Alnus nepalensis) is often used as a shade tree (Chempakam and Sindhu, 2008). The plant can grow at altitudes ranging from 600 to 2000 m above mean sea level and in areas with an annual rainfall varying from 2800 to 3500 mm (Sharma *et al.*, 2000). The useful portion of large cardamom is the dried capsule, which has 40 to 50 small seeds and is grayish brown to dark red brown. The capsules are held together inside the spike with viscous sugary pulp and are 20–25 mm long and oval to globule in shape (Thomas *et al.*, 2009).

Large cardamom pods can be used as a whole or in split in some of the south Asian (mostly Nepalese and Indian) cuisines (Khare *et al.* (2012). It is incorporated in sweet dishes, drinks like punches and mulled wines, milk tea, curries, different rice dishes, pickles etc. Large cardamom also possesses curative properties, and been one of the major spices which are mentioned in Ayurveda and Unani medicine (Chempakam and Sindhu, 2008). From pre-historic time, large cardamom seeds have been used to cure dyspnoea, cough, thirst, vomiting, disease of mouth, nausea, itching, indigestion, biliousness, abdominal pains and rectal diseases in local villages and cities (Gopal *et al.*, 2012). It is believed that cleaned, sound, free from fungal growth or foreign material with bold and uniform sized cardamom capsules having dark pink colorations have high commercial values in the market (Mande *et al.*, 1999). Cardamom capsules and seeds are used to treat gonorrhea, congestive jaundice, headache, and stomatitis and to control insects (Satyal *et al.*, 2012).

2.5.5 *Elettaria cardamomum* (Small Cardamom)

Small cardamom, known as the 'queen of spices', which belongs to the family of Zingiberaceae, is a rich spice obtained from the seeds of a perennial plant, Elettaria cardamomum Maton. It is one of the highly prized spices of the world and is the third most expensive spice after saffron and vanilla (Mehra, 2001). Cardamom is a perennial bushy herb, growingto about 4.5 m (15 ft) with mauve-marked, orchid-like white flowers and very long, lance-shaped leaves (Chempakam and Sindhu, 2008).

The major use of cardamom is for domestic culinary purposes. The spice is used in the form of the whole fruit, the decorticated seeds, or the ground seeds. In Asia, cardamom plays an important role in a variety of spiced rice, vegetable and meat dishes. It is used to flavour coffee and tea and is an important ingredient of curries. In the food and beverage industry it is used for flavouring confectionery, a range of baked goods, prepared savoury dishes, and a range of beverages. Locally it is a masticatory often included in the betel quid, and industrially it is used to a small extent in flavouring tobacco. Cardamom is included in several pharmacopoeias. It is considered tonic to the heart, stomachic, laxative, diuretic, and carminative. It lessens inflammation, headache, earache, toothache, and alleviates disorders of the liver, chest and throat. Cardamom is commonly given in instances of snake bite and scorpion sting, but it is not an antidote (Wardini and Thomas, 2016).

Part III

Materials and methods

3.1 Materials

3.1.1 Spices

Spices like cinnamon (*Cinnamomum zeylanicum* Cassia), cloves (*Syzygium aromaticum* Myrtaceae), timur (*Zanthoxylum aramatum* planispinum), large cardamom (*Amomum subulatum* Roxb.) and small cardamom (*Elettaria cardamomum* Malabar) were brought from local market of Dharan.

3.1.2 Chemicals and equipment

All chemicals, glassware and equipment required were used from the laboratory of Central Campus of Technology, Dharan. Details of chemicals and equipment are given in Table 3.1 and Table 3.2 respectively.

Table 3.1 Chemicals

Name of chemical	Manufacturer	Assay
DPPH	HiMedia Laboratories Pvt. Ltd.	97 %
Folin-ciocalteu reagent	Thermo Fischer Scientific , India	2 N
Gallic Acid	HiMedia Laboratories Pvt. Ltd.	98 - 102 %
Sodium Carbonate	Qualigens Fine Chemicals	99.5 %
Methanol	Merck Life Science Pvt. Ltd.	99 %
Acetic acid	Thermo Fischer Scientific , India	99.5 %
Chloroform	Merck Life Science Pvt. Ltd.	99 %
Quercetin	HiMedia Laboratories Pvt. Ltd.	99.5 %

Table 3.2 Materials

Physical Apparatus	Physical apparatus
Electric balance	Spectrophotometer
Whatmann filter paper	Micropipette
Incubator	Pipette
Glasswares (Beaker, Test tubes, Volumetric flask, Conical Flask, burette, Beaker)	Measuring cylinders

3.2 Methods

3.2.1 Preparation of plant extracts

Plant materials were extracted as per Ahmad *et al.* (2014) with slight modification.10 g of powdered spices were steeped in 80% methanol (100 ml) for 12 h at room temperature. They were then filtered using Whatman No.1 filter paper. Finally, extracts were transferred to brown coloured glass bottles, sealed by using bottle caps and stored at 4 ± 2ºC until analysis. The extract concentration was determined by evaporating 5 ml of extract (at 80ºC) to dryness and measuring the weight.

3.2.2 Preliminary test of phytochemicals

The phytochemical content of plant leaves were analyzed according to the standard procedures as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

3.2.2.1 Total phenol

The extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% Ferric Chloride ($FeCl_3$) solution was added. A dark green color indicated the presence of phenolic compounds.

3.2.2.2 Flavonoid

2ml of 1% ammonia solution was added to the aqueous filtrate of sample in a test tube. A yellow coloration observed indicated the presence of flavonoids.

3.2.2.3 Tannin

2ml of distilled water was added to 1ml of plant extract in a test tube. 1ml of 0.1% Ferric Chloride ($FeCl_3$) reagent was then added. A blue black precipitate was taken as an evidence for the presence of tannins.

3.2.2.4 Saponin

About 2 g of the crushed plant sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. No frothing was evident.

3.2.2.5 Alkaloid

5 g of crushed sample was stirred with 5 ml of 1% Hydrochloric acid (HCL) on a steam bath. 5 ml of Mayer's (1.35 g mercuric chloride in 60 ml water + 5 g potassium iodide in 10 ml water) and then observed. No yellow colored precipitate was evident for the presence of alkaloids.

3.2.3 Determination of total phenolic content

Total phenol content was determined as described by Waterhouse (2002). 0.5 ml of the extract was taken in which 2.5 ml of 10 % Folin-Ciocalteu reagent was added, then 2.5 ml of 10 % sodium carbonate was added.Then the prepared solution was incubated for 30 min at 45ºC and then the absorbance was taken at 765 nm against a reagent blank. Gallic acid was taken as standard and the results was expressed as mg of gallic acid equivalents (GAEs) per g of the dried extracts.

3.2.4 Determination of total flavonoid content

Total flavonoid content was determined using a modified aluminium chloride assay method as described by Barek and Hasmadi (2015). 2 ml of solution was pipette out in a test tube in which 0.2 ml of 5% Sodium Nitrate ($NaNO_3$) was mixed and stand for 5 min. 0.2 ml Aluminium Chloride ($AlCl_3$) was pipetted out, mixed in the tube and allowed to stand for 5 min. This followed addition of 2 ml of 1N Sodium Hydroxide (NaOH) in the tube and finally volume was made up to 5ml. The absorbance was measured after 15 min at 510 nm against a reagent blank. Quercetin was taken as standard and the results was expressed as mg of Quercetin equivalents (QE) per g of the dried extracts.

3.2.5 Determination of tannin content

Total tannin content was determined by Folin-Ciocalteu method as described by Mythili *et al.* (2014). 0.1 ml sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent. 1 ml of 35 % Na_2CO_3 solutions and diluted to 10 ml with distilled water The mixture was shaken well and left at room temperature for 30 min. Gallic acid was taken as standard and the results was expressed as mg of gallic acid equivalents (GAEs) per g of the dried extracts.

3.2.6 Free radical scavanging capacity

DPPH free radical scavenging activities (antioxidant activities) of extracts were determined by method described by Vignoli *et al.* (2011) with slight variation. Different dilutions of the extracts were made using 80% methanol. Then 1 ml of the extract was mixed with 2 ml of 0.1 mM DPPH solution. The absorbance was read at 517 nm after 30 min incubation in the dark. Finally, percentage scavenging activity was determined using following equation

$$\% \text{ scavenging activity} = (Ac-As) \times 100 / Ac$$

Where Ac is the absorbance of control and As is the absorbance of test sample.

3.2.7 Formulation of spiced mixture

The spiced mixture were formulated as M1, M2, M3, M4 and M5 and A, B, C, D and E represents Cinnamon, cloves, timur, large cardamom and small cardamom respectively which is shown in Table 3.3.

Table 3.3 Formulation of Spiced mixture

Spiced Mixture	A	B	C	D	E
M1	0.1	0.1	0.1	0.1	0.6
M2	0.1	0.1	0.1	0.6	0.1
M3	0.1	0.1	0.6	0.1	0.1
M4	0.1	0.6	0.1	0.1	0.1
M5	0.6	0.1	0.1	0.1	0.1

Part IV

Results and discussions

Sample for this study includes cinnamon (*Cinnamomum zeylanicum Cassia)*, cloves (*Syzygium aromaticum* Myrtaceae), timur (*Zanthoxylum aramatum* Planispinum*)*, large cardamom (*Amomum subulatum* Roxb.*)* and small cardamom (*Elettaria cardamomum* Malabar*)* collected from local markets of Dharan and their mix was also prepared which were used to test the presence of phytochemicals

4.1 Phytochemical screening

During the experimental work, the methanolic extract of spices have shown to have the total phenol, total flavonoid, tannin and antioxidant activity which is shown in Table 4.1.

Table 4.1 Phytochemical screening

S.N	Phytochemicals	Cinnamon	Cloves	Large cardamom	Small cardamom	Timur
1.	Phenols	+ ve	+ ve	+ ve	+ ve	+ ve
2.	Flavonoids	+ ve	+ ve	+ ve	+ ve	+ ve
3.	Tannins	+ ve	+ ve	+ ve	+ ve	+ ve
4.	Alkaloids	- ve	- ve	- ve	- ve	- ve
5.	Saponins	- ve	- ve	- ve	- ve	- ve

The phytochemical screening of methanolic extract of spices showed that they were rich in flavonoids, tannins, phenols and also possess antioxidant properties as well. According to Chu and Chen (2006), polyphenols are the powerful antioxidant in plants which modify body's reactions to show anticancer, anti-inflammatory, antimicrobial and anti-allergic activity and thus exhinit antioxidant activity (Balch, 2000).

4.2 Total phenol content

Total phenol contents of methanolic extracts of spices are shown in Table 4.2. The total phenol contents of spices extracts were found to be significantly different ($p < 0.05$).

Table 4.2 Total phenol content of methanolic extracts of spices

S.N.	Spices samples	Total phenol content (mg GAE/g)*
1.	Cinnamon	$158.14^{a} \pm 1.07$
2.	Cloves	$149.59^{b} \pm 5.28$
3.	Timur	$142.77^{c} \pm 1.04$
4.	Large cardamom	$92.81^{d} \pm 3.93$
5.	Small cardamom	$74.70^{e} \pm 3.20$

*Values are the means of three determinations and the figures in the parentheses are their standard deviation. Means with different superscripts are different ($p < 0.05$).

Phenolic compounds in plants provide an array of natural sources of antioxidants for use in foods and nutraceuticals (Shahidi, 1992). Experimental studies strongly support a role of polyphenols in the prevention of cardiovascular disease, cancer, osteoporosis, diabetes mellitus and neurodegenerative disease (Scalbert *et al.,* 2005). Thus, higher content of these components are anticipated to be beneficial for health.

The highest content of phenol was found in cinnamon followed by cloves, timur, large cardamom and small cardamom as shown in Table 4.2. Su *et al.* (2005) reported total phenol content in crude extract (in 80% methanol) of cinnamon bark to be 148 mg GAE/g dry matter which is similar to our findings. The values for total phenolic content in cloves in our study is little lower compared to that previously reported (Adaramola and Onigbinde, 2016). The differences in the phenol contents may be due to the variation in species, method of analysis, harvesting time and climatic conditions of growing area.

4.3 Total flavonoid content

The total flavonoid contents of methanolic extracts of spices are shown in Table 4.3.

Table 4.3 Total flavonoid content of methanolic extracts of spices

S.N.	Spices samples	Total flavonoid content (mg Quercetin /g)*
1	Cinnamon	$225.31^{d} \pm 10.59$
2	Cloves	$337.29^{c} \pm 10.59$
3	Timur	$76^{b} \pm 2.93$
4	Large cardamom	$84.49^{b} \pm 8.81$
5	Small cardamom	$55.64^{a} \pm 5.87$

*Values are the means of three determinations and the figures in the parentheses are their standard deviation. Means with different superscripts are different (p < 0.05).

Flavonoids exert their antioxidative activity by effectively scavenging various free radicals (like superoxide anion and peroxynitrite), by regulating oxidative stress-mediated enzyme activity (Ramos, 2008) and by chelation of transition metals involved in radical forming processes (Biesaga, 2011). Flavonoids can interfere with all three stages of cancer: the initiation, development and progression by modulating cellular proliferation, differentiation, apoptosis, angiogenesis as well as metastasis (Kris-Etherton *et al.*, 2002). Moreover, chemopreventive effect of dietary flavonoids is quite specific as cancerous cells have shown to be more sensitive to polyphenol actions than normal cells. Furthermore, flavonoids exhibit antibacterial, anti-inflammatory, anti-allergic and anti-thrombotic actions (Lewandowska *et al.*, 2015).

From our analysis it was found that the flavonoid content of timur and large cardamom are not significantly different and the flavonoid content of rest of the spices are significantly different (p < 0.05).The highest content of flavonoid was found in cloves followed by cinnamon, large cardamom, timur and small cardamom.

Adaramola and Onigbinde (2016) reported that the total flavonoid content in crude extract (in 80% methanol) of cloves to be 318.67±0.88 mg QE/g which is similar to our finding. Vavaiya *et al.* (2012) reported the total flavonoid content of 24.8 mg QE/ g during the study of large cardamom.The differences in the flavonoid contents may be due to the variation in species, method of analysis, harvesting time and climatic conditions of growing area.

4.4 Total tannin content

Total tannin content of methanolic extracts of spices are shown in Table 4.4. The tannin content of all five spices extracts were found to be significantly different ($p < 0.05$).

Tannins are inhomogeneous class of biochemical compounds that are found in almost all plant families. They provide astringent and unpleasant taste and hence not much valued in Spice Plants. High tannin content is considered as bad quality (e.g., cassia) and in very small levels has some culinary merits (rosemary, sumac) (Sunder, 2016).

Table 4.4 Total tannin content of methanolic extract of spices

S.N.	Spices sample	Total tannin content (mg GAE/g)*
1.	Cinnamon	$15.47^{e} \pm 0.13$
2.	Cloves	$14.06^{d} \pm 0.49$
3.	Large cardamom	$12.88^{c} \pm 0.54$
4.	Timur	$11.47^{b} \pm 0.27$
5.	Small cardamom	$10.38^{a} \pm 0.27$

*Values are the means of three determinations and the figures in the parentheses are their standard deviation. Means with different superscripts are different ($p < 0.05$).

From our analysis it was found that the highest tannin content was found in cinnamon followed by cloves, large cardamom and small cardamom. The tannin content in spices ranged from 0.6 to 8 mg GAE/g (Ugwuona, 2014). Kumari and Jain (2015) reported that the total tannin content of raw cinnamon bark to be 3.46 mg/g which is less than our finding. This may be because of drying of raw cinnamon bark as Obeta (2015) reported that the drying of *Vernonia amygdalina* and *Gongronema latifolium* leaves increases it's tannin content.

4.5 Total antioxidant activity

Total antioxidant activity of methanolic extracts of spices are shown in Table 4.5. The activity of spices extracts were found to be significantly different ($p < 0.05$). However, the antioxidant activity of Timur and Large Cardamom was not significantly different.

Table 4.5 Total antioxidant activity of methanolic extracts of spices

S.N.	Spices sample	Total antioxidant activity (%)
1	Cinnamon	$68.74^{d} \pm 1.56$
2	Cloves	$60.15^{b} \pm 2.34$
3	Timur	$64.58^{b} \pm 0.90$
4	Large cardamom	$65.62^{c} \pm 1.56$
5	Small cardamom	$50^{a} \pm 1.56$

*Values are the means of three determinations and the figures in the parentheses are their standard deviation. Means with different superscripts are different ($p < 0.05$).

From our analysis it is seen that the highest antioxidant activity is shown by cinnamon followed by large cardamom, timur, cloves, and small cardamom. Filho *et al.* (1999) reported the total antioxidant activity of cinnamon to be 95.5 %. The difference in the result may be due to the variation in species and method of analysis.

Antioxidants are reported to protect cells from the damage of reactive oxygen species (ROS). ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease. Moreover, the initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system (Lobo *et al.*, 2010).

Mashkor (2015) reported the total antioxidant of cloves to be 68.70 %. Khare *et al.* (2012) reported that the Total antioxidant activity of large cardamom to be 41.2 % which is less than our finding. The difference in the result may be due to variation in solvent for extraction.

The extraction yield of pure methanol is higher than that of pure ethanol and pure acetone and the extraction yield increases with increasing polarity of the solvent used in extraction (Angkawijaya *et al.*, 2014).

4.6 Phytochemical and antioxidant activity of spiced mixture

The phyotochemicals and antioxidant activity of spiced mixture is given in Table 4.6. The phytochemical and antioxidant activity of all five spiced mixture were found to be significantly different (p < 0.05).

Table 4.6 Phytochemical and antioxidant activity of spiced mixture

S.N	Spiced mixture	Total phenol content	Total flavonoid content	Total tannin content	Antioxidant activity
1.	M1	$98.98^{a} \pm 0.17$	$105.78^{a} \pm 0.40$	$11.52^{a} \pm 0.09$	$55.40^{a} \pm 3.59$
2.	M2	$107.96^{b} \pm 0.58$	$119.78^{b} \pm 0.31$	$12.78^{b} \pm 0.18$	$63.76^{b} \pm 1.67$
3.	M3	$132.91^{c} \pm 0.50$	$115.26^{c} \pm 0.60$	$12.24^{c} \pm 0.08$	$63.17^{c} \pm 1.93$
4.	M4	$136.13^{d} \pm 0.59$	$246.01^{d} \pm 0.62$	$13.37^{d} \pm 0.45$	$60.88^{d} \pm 2.45$
5.	M5	$140.21^{e} \pm 0.86$	$190.46^{e} \pm 0.80$	$14.31^{e} \pm 0.20$	$65.34^{e} \pm 0.16$

*Values are the means of three determinations and the figures in the parentheses are their standard deviation. Means with different superscripts are different ($p < 0.05$).

From our analysis, it was found that M5 spiced mixture having cinnamon 0.6 parts was found to be optimized mixture as it has highest phytochemical (Phenol, flavonoid and tannin) and antioxidant activity. Basu *et al.* (2016) reported the total phenol and antioxidant activity of Garam Masala to be 145 mg GAE/g and 70 % respectively which is similar to our finding.

Part V

Conclusions and recommendations

5.1 Conclusions

The crude methanolic extracts of spices were analysed for total phenol contents, total flavonoid content, total tannin content and DPPH free radical scavenging activities. Based on the results following conclusions were drawn.

1. Phenol, flavonoid and tannins were detected in all five spices.
2. The highest phenol content was found in cinnamon followed by cloves, timur, large cardamom and small cardamom.
3. The highest flavonoid content was found in cloves followed by cinnamon, large cardamom , timur and small cardamom.
4. The highest tannin content was found in cinnamon followed by cloves, large cardamom, timur and small cardamom.
5. All the Spices were found to have free radical scavenging (antioxidant) activities. Among the spices analysed, cinnamon was found to have higher antioxidant activity.
6. Among the spiced mixture analysed, spiced mixture having cinnamon 0.6 parts and rest spices 0.1 parts was found to be optimized mixture.

5.2 Recommendations

1. Antimicrobial activity of spices can also be studied.
2. Composition of crude extract can also be studied.
3. Comparative study of phytochemical and antioxidant activity in different organic solvent can also be studied.

Summary

Spices can be used as medicine because they are natural products easily absorbed by our bodies and generally do not have any adverse effects. Spices do more than add a dash of heat or flavor to your meals. Folk remedies and traditional medicine like ayurveda, naturopathy, and herbal medicine have long used these kitchen staples to treat ailments, boost immunity, and maintain general health.

Phytochemicals like phenols, flavonoids, tannins, etc are naturally present in the plants and shows biologically significance by playing an essential role in the plants to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. There is evidence from laboratory studies that phytochemicals in Spices may reduce the risk of cancer and have antibacterial, antifungal, antioxidative, hormonal action, enzyme stimulation and many more, possibly due to dietary fibers, polyphenol antioxidants and anti-inflammatory effects.

Thus, in the current study spices like cinnamon, cloves, timur, large cardamom and small cardamom were bought from local markets of Dharan. These spices were steeped in 80% methanol for 12 h at room temperature. Then the extracts were filtered using Whatman No. 1 filter paper, transferred to brown bottle and stored at refrigerated temperature until analysis. The analysis was made for total phenol content, total flavonoid content, total tannin content and DPPH free radical scavenging activities.

The qualitative preliminary phytochemical analysis of the spices was performed using 80% methanol to detect the presence of phyto-constituents. The methanol extract showed the presence of tannin, flavonoid and polyphenol and antioxidant activity as well. The highest phenol content was found in cinnamon followed by cloves, timur, large cardamom and small cardamom. Similarly, the highest flavonoid content was found in cloves followed by cinnamon, large cardamom, timur and small cardamom. The highest tannin content was found in cinnamon followed by cloves, large cardamom, timur and small cardamom. The highest antioxidant activity is shown by cinnamon followed by large cardamom, timur, cloves and small cardamom. Among the spiced mixture analysed, Spiced mixture having cinnamon 0.6 parts and rest spices 0.1 parts was found to be optimized mixture.

The results of this work could be useful for functional food and pharmacological industries. Identification of spices with higher antioxidant activities is not only beneficial for pharmacological industries and the natives using such spices but also contribute to the transfer of highly specialized knowledge of spices to the world.

References

FDA. (1992). FAQS on food additives-1992.

Adaramola, B. and Onigbinde, A. (2016). Effect of extraction solvent on the phenolic content, flavonoid content and antioxidant capacity of clove bud. *J. Pharm. and Biol. Sci.* **11** (3), 33-38.

Ahmad, W., Khan, I., Khan, M. A., Ahmad, M., Subhan, F. and Karim, N. (2014). Evaluation of antidiabetic and antihyperlipidemic activity of *Artemisia indica* linn (aeriel parts) in streptozotocin induced diabetic rats. *J. Ethnopharmacol.* **151**, 618-623.

Ali, A. and Alqurainy, F. (2006). Activities of antioxidants in plants under environmental stress. *Afr. J. Biotechnol.*, 187-256.

Allen, J. C. and Hamilton, R. J. (1983). Lipid oxidation. *In:* "Rancidity in Foods". (J. B. Rossell, Ed.). London, New York. Applied Sci. Pub.

Angkawijaya, A. E., Do, Q. D., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S. and Ju, Y. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J. Food and Drug Anal.* **296-302**.

Anonymous. Crystal Quinone. Retrieved from http://www.crystalquinone.com/TBHQ.htm. [Accessed 15th August 2010].

Arques, J. L., Rodriguez, E., Nunez, M. and Medina, M. (2008). Inactivation of gram negative pathogens in refrigerated milk by reuterin in combination with nisin or the lactoperoxidase system. *Eur. Food Res. Tech.* **227** (1), 77-82.

Azwanida, N. (2015). A review on the extraction methods used in medicinal plants, principle, strength and limitation. *J. Med. and Aromatic plants*. **4** (2167-0412), 196.

Balch, J. F. (2000). "Prescription for Nutritional Healing" (5th ed.). Pengium Puntam Inc.

Baral, S. R. and Kurmi, P. P. (2006). "A Compendium of Medicinal Plants in Nepal". Kathmandu. Nepal.

Barek, M. L. and Hasmadi, M. (2015). Effect of different drying methods on phytochemicals and antioxidant properties of unfermented and fermented teas from Sabah snake grass (*Clinacanthus nutans* Lind.) leaves. *Int. Food Res. J.* **22** (2), 661-670.

Barkatullah, M., Muhammad, N., Khan, N., Khan, S. A., Zafar, S., Jan, S., Riaz, N., Ullah, Z., Farooq, U. and Hussain, J. (2017). Pharmacognostic and phytochemical studies of *Zanthoxylum aramatam* DC. *Pak. J. Pharm. Sci.* **30** (2), 429-438.

Basu, T., Bhattacharyya, S., Maitra, S. and Rai, C. (2016). Evaluation of antioxidant and antimicrobial activities of Garam Masala, an Indian spice blend, and its' components in- vitro. *Der Pharmacia Lettre*. **8** (20), 110-116.

Bauernfeind, J. C. and Cort, W. M. (1974). Tocopherols. *In:* "Encyclopedia of Food Technology and Food Science Serie". (B. Caballero, Ed.). pp. 16-74. Westport. AVI Pub. Co.

Bellomo, G. (1991). Cell demage by oxygen free radicals. *In:* "Cytotechnology" (Vol. 5). (D. W. Barnes, S. Shirahata and Y. Katakura, Eds.). pp. 71-73. Kluwer Academic Publishers.

Bhattacharya, D. K. (2003). Antioxidants in oils and fats: Some technical aspects. Presented at Ic-antioxidant-03. Kolkata and Jadavpur Univ. . July 15-16. p. 40.

Biesaga, M. (2011). Influence of extraction methods on stability of flavonoids. *J. Chromatogr.* **1218**, 2505-2512.

Birt, D. A. (2006). Phytochemicals and cancer prevention: from epidemiology to mechanism of action. *J. Am. Diet. Assoc.* **106**, 20-24.

Bishov, S. J. and Henic, A. S. (1977). Natural antioxidants. *Enc. food Sci.* **45**, 299.

Bisht, V. K., Negi, A., Bhandari, A. K. and Sundriyal, R. C. (2011). *Amomum subulatum* Roxb.: Traditional, phytochemical and biological activities—an overview. *J. Agric. Res.* **6** (24), 5386–5390.

Blumenthal, M., Busse, W. R., Goldberg, A., Gruenwald, J., Hall, T., Riggins, C. W. and Rister, R. S. (1998). "Therapeutic Guide to Herbal Medicines". American Botanical Council. Austin.

Bradlaw, H., Telang, N., Seplovic, D. and Osborne, M. (1999). Phytochemicals as modulators of cancer risks. *Adv. Exp. Med. Biol.* **472**, 207-221.

Briskin, D. P. (2000). Medicinal plants and phytochemicals. *Plant Physiol.* **124**, 507-514.

Burns, J. P. T., Gardner, D., Mathew, G. G., Duthie, M., Lean, E. and Crozier, A. (2001). Extraction of phenolic and changes in antioxidant activity of red wine during vinification. *J. Agric. and Food Chem.* **49**, 5797-5808.

Burr, G. O. and Burr, M. M. (1929). Progress in the chemistry of fats and other lipids. *J. Biol. Chem.* **9** (4), 556-565.

CAC. (2014). "Code of Hygienic Practices for Spices and Dried Aromatic Plants". Codex Alimentarius Commission.

Chandra, S. T. and Goyal, A. (2014). Antioxidant activity by DPPH radical scavenging method America. *J. Ethnomed.* **1**, 244-249.

Chang, S. S., Ostric Matijasevic, B. and Hsieh, O. A. (1977). Natural antioxidants from rosemary and sage. *J. Food sci.* **42**, 1102.

Chempakam, B. and Sindhu, S. (2008). Large cardamom. *In:* "Chemistry of Spices". (V. A. Parthasarathy, B. Chempakam and T. J. Zachariah, Eds.). pp. 60-69. Wallingford, UnitedKingdom. CAB International.

Chipault, J. R. (1862). Antioxidants for use in foods. *In:* "Autoxidation and Antioxidants" (Vol. 2). (W. O. Lundberg, Ed.). p. 477. New York.

Chitravadivu, C., Manian, S. and Kalaichelvi, K. (2009). Qualitative analysis of selected medicinal plants. *J. Sci. Res.* **4** (3), 144-146.

Chu, S. C. and Chen, C. (2006). Effect of origin and fermentation time on the antioxidants activities of kombucha. *J. Food Chem.* **3**, 2619- 2623.

Coppen, P. P. (1983). "Rancidity in foods". Applied Sci. Pub. London and New York.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12** (4), 564-582.

Crawford, P. (2009). Effectiveness of cinnamon for lowering hemoglobin A1C in patients with type 2 diabetes: a randomized, controlled trial. *J. Am. Board Fam. Med.* **22** (5), 507-512.

De, M., De, A. K. and Banerjee, A. B. (1999). Antimicrobial screening of some Indian spices. *Phytotherapy Res.* **13**, 616-618.

Dolara, P., Luceri, C., Femia, A. P., Giovanelli, L., Carderni, G., Cecchini, C., Silvi, S., Orpianesi, C. and Cresci, A. (2005). Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutation Res.* **591**, 237-246.

Doss, A. and Anand, S. P. (2012). Preliminary phytochemical screening of *Asteracantha longifolia* and *Pergularia daemia*. *World App. Sci. J.* **18** (2), 233-235.

Dutton, H. J., Schwab, A. W. and Moser, H. A. (1949). Bailey's industrial oils and fat products. *J. Am. oil chemists' soc.* **3**, 303.

Farrel, K. T. (1990). "Spices, Condiments and Seasonings" (2 ed.). AVI book, Van Nostrand Reinhold. Newyork.

Filho, J. M., Van-Koiij, A., Mancini, D. P. and Torres, R. P. (1999). Antioxidant activity of cinnamon (*Cinnamomum Zeylanicum*, Breyne) extracts. *Bollettino Chimico Farmaceutico*. **137** (11), 443-447.

Furia, T. E. (1968). "Handbook of Food Additive". Vol. 3.

Gaur, R. D. (1999). Flora of the district Garhwal, North West Himalaya: with ethnobotanical notes. *Transmedia*. 811.

Geweli, M. B. and Awale, S. (2008). "Aspects of Traditional Medicine in Nepal". Institute of Natural Medicines University of Toyama. Japan.

Ghasemzadeh, A., Jaffar, H. and Rahmat, A. (2010). Antioxidant activities, total phenolics and total flavonoid content in two varieties of Malasiya young ginger *Molecules*. **15**, 4324-4333.

Gopal, K., Baby, C. and Mohammed, A. (2012). *Amomum subulatum* Roxb: An overview in all aspects. *Int. Res. J. Pharm.* **3** (7), 96-99.

Grieve, M. (1931). A Modern Herbal. Dover Publications, Inc. Retrieved from http://botanical.com/botanical [Accessed 20 January, 2018].

Gulcin, I., Kufrevioglu, O. L., Oktay, M. and Guyukokuroglu, M. E. (2004). Antioxidant, anticancer, antiulcer and analgesic activities of nettle (*Urtica dioica* L). *J. Ethnopharmacol.* **90**, 205-215.

Gyamfi, M. A. and Aniya, Y. (2002). Antioxidant properties of thonningianin A, isolated from the African medicinal herb, *Thonningia sanguine*. **63**, 1725-1737.

Halliwell, B. and Gutteridge, J. M. C. (1986). "Free Radicals in Biology and Medicine". Clarendon Press. Oxford, UK.

Handa, S., Khanija, S., Longo, G. and Rakesh, D. (2008). "Extraction Technologies for Medicinal and Aromatic Plants" (1st ed.). United Nations Industrial Development Organization. Italy.

Harborne, J. and Baxter, H. (2000). "The Handbook of Natural Flavonoids" (2 ed.). Vol. 1. John Wiley and Sons. Chichester.

Harborne, J. B. (1973). "Phytochemical Methods" (1 ed.). Vol. 2. Chapman and Hall Ltd. London.

Haslam, E. (1989). "Plant Polyphenols". Cambridge University Press. Cambridge.

Hayes, D. P. (2005). The protective role of fruits and vegetables against radiation – induced cancer. *Nutr. Rev.* **63**, 303-322.

Hertog, M. G. L., Hollman, P. C. H. and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and fruits commonly consumed in the Netherlands. *J. Agri. Food Chem.* **40**, 2379-2383.

Hinneburg, I., Dorman, H. J. D. and Hiltunen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. *Food chem.* **97**, 122-129.

Imida, K., Fukushima, S., Shirai, T., M., O., Nakanishi, K. and Ito, N. (1983). "Interactions of Food Components" (G.G. Birch and M.G. Lindley ed.). Elsevier applied science pub. London and New York.

Jakhetia, V., Patel, R., Khatri, P., Pahuja, N., Garg, S., Pandey, A. and Sharma, S. (2010). Cinnamon: a pharmalogical review. *J. Adv. Sci. Res.* **1** (2), 19-23.

Jyothiprabha, V. and Venkatachalam, P. (2016). Preliminary phytochemical screening of different solvent extracts of Selected lndian spices. *Int. J. Current Microbiol. and Appl. Sci.* **5**, 116-122.

Kaefer, C. M. and Milner, J. A. (2008). The role of herbs and spices in cancer prevention. *J. Nutr. biochem.* **19** (6), 347-361.

Kamatou, G. P., Vermaak, I. and Viljoen, A. M. (2012). Eugenol-from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule. *Molecules*. **17** (6), 6953-6981.

Katzer, G. (2014). Cloves (*Syzygium aromaticum* [L.] Merr. et Perry) Spice Pages. Retrieved from http://gernot-katzers-spice-pages.com/engl/Syzy_aro.html [Accessed 20 January, 2018].

Kelly, E. H., Anthony, R. T. and Dennis, J. B. (2002). Flavonoid antioxidants: chemistry, metabolism, and structure activity relationship. *J. Nutri. Biochem.* . **13** (10), 572-584.

Khalsa Singh, K. P. and Tierra, M. (2008). The way of Ayurvedic herbs. Twin Lakes: Lotus Press.

Khare, D. P., Brajesh, K., Hussain, A., Verma, S. and Mishra, M. (2012). Evaluation of antioxidant activity of large cardamom (leaves of *Amomum subulatum*). *Int. J. Drug Dev. Res.* **4** (1).

Kim, S. H., Hyun, S. H. and Choung, S. Y. (206). Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. *J. Ethnopharmacol.* **104**, 119-123.

King, A. and Young, G. (1999). Characterisation and occurrence of phenolic phytochemicals. *Proc. Nutr. Soc.* **45**, 415-419.

Kirk, S. and Sawyer, R. (1991). "Pearson's Composition and Analysis of Foods". Longman Group Ltd. UK.

Krinsky, N. I. (1992). Mechanism of action of biological antioxidants. *Proc. Soc. Exp. Biol. Med.* **200**, 248.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., Griel, A. E. and Etherton, T. D. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* . **113**, 71-88.

Kumar, S. and Pandey, A. K. (2013). Chemistry and biolodical activities of flavonoids: An overview. *The Scientific World J.*, 16.

Kumari, M. and Jain, S. (2015). Screening of potential sources of tannin and its therapeutic application. *Int. J. Nutr. Food Sci.* **4** (2-1), 26-29.

Lee, F. A. (1975). "Basic Food Chemistry". The AVI Publishing Company, Inc. Westport.

Lesschaeve, I. and Noble, A. C. (2005). Polyphenols: factors influencing their sensory properties and their effect on food and beverage preference. *Am. J. Clin. Nutr.* **81**, 3305-3355.

Lewandowska, H., Kalinowska, M., Lewandowski, W., Epkowski, T. M. and Brzóska, K. (2015). The role of natural polyphenols in cell signaling and cytoprotection against cancer development. *J. Nutr. Biochem.* **32**, 1-19.

Lobo, V., Patil, A., Pathak, A. and Chandra, N. (2010). Free radicals,antioxidants and functional foods: Impacts on human health. *Phcog Rev.* **4**, 118-126.

Lugemwa, F., Snyder, A. and Shaikh, K. (2013). Determination of radical scavenging activity and total phenols of wine and spices: A randomized study. *Antioxidants*. **2**, 110-121.

Manach, C., Scalbert, A., Morand, C., Remesy, C. and Limenez, L. (2004). Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **79**, 727-747.

Mande, S., Kumar, A. and Kishore, V. V. N. (1999). A study of large cardamom curing chambers in Sikkim. *Biomass Bioenergy*. **16**, 463-473.

Mang, B., Wolters, M., Schmitt, B., Kelb, K., Lichtinghagen, R., Stichtenoth, D. O. and Hahn, A. (2009). Effects of a cinnamon extract on plasma glucose, HbA, and serum lipids in diabetes mellitus type 2. *Eur. J. Clin. Invest.* **36** (5), 340-344.

Mashkor, I. M. (2015). Evaluation of antioxidant activity of clove (*Syzygium aromaticum*). *Int. J. Chem. Sci.* **13** (1), 22-30.

Matan, N., Rimkeeree, H., Mawson, A. J., Chompreeda, P., Haruthaithanasan, V. and Parker, M. (2006). Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. *Int. J. Food Microbiol.* **107** (2), 180-185.

McCord, J. M. (2000). The Evolution of Free Radicals and Oxidative Stress. *Am. J. Med.* **108**, 659.

Mehra, K. (2001). Indian system of innovation in biotechnology – a case study of cardamom. *Technovation*. **21** (1), 15-23.

Meyer, L. H. (1987). "Food Chemistry". CBS, Publishers and Distributors. Delhi, India.

Mythili, K., Reddy, C. U., Chamundeeswari, D. and Manna, P. K. (2014). Determination of total phenol, alkaloid, flavonoid and tannin in different extracts of *Calanthe Triplicata*. *J. Pharmaco. Phytochem.* **2** (2), 2.

O' Connell, J. E. and Fox, P. F. (2001). Significance and applications of phenolic compounds in the production and quality of milk and dairy product. *Int. Dairy J.* **11**, 103-120.

Obeta, N. A. (2015). Effect of moist heating and drying processing on the proximate and phytochemical composition of *Vernonia amygdalina* and *Gongronema latifolium* leaves. *Agric. Sci. Res. J.* **5** (11), 153-165.

Oboh, G. (2006). Antioxidant properties of some commonly consumed and underutilized tropical legumes. *Eur. Food Res. Technol.* . **224**, 61-65.

Okadu, T. and Ito, H. (2013). Tannins of constant structure in medicinal and food plants, hydrolyzable tannins and polyphenols related to tannins. *Molecules*. **16**, 1420-3049.

Okpekon, T., Yolou, S., Gleye, C., Roblot, F., Loiseau, P., Bories, C., Grellier, F., Frappier, A., Laurens, A. and Hocquemiller, R. (2004). Antiparasitic activities of medicinal plants used in Ivory Coast. *J. Ethnopharmacol.*, 91-97.

Palavy, k. and Priscilla, M. D. (2006). Standardisation of selected Indian medicinal herbal raw materialcontaining polyphenols as major constituents. *J. Pharma. Sci*. **68**, 506-509.

Pokorny, J. (2007). "Antioxidants in Food Preservation" (2 ed.). CRC Press. New York.

Prasad, M. P., Shekhar, S. and Babhulkar, A. (2012). Phytochemical analysis and antioxidant potential of piper species and its molecular characterization by RAPD marker. *Int. J. Fundamental Appl. Sci.* **1** (2278-1404), 71-73.

Pratt, D. E. and Watts, B. M. (1964). The antioxidant activity of vegetable extracts. *J. sd. Sci.* **29** (27).

Province, R. H. (1978). "Barefoot Doctor's Manual ". Routledge & Kegan Paul PLC.

Rajbhandari, K. R. (2001). "Ethnobotany of Nepal" (189 ed.). Ethnobotanical society of Nepal. Kathmandu.

Ramanthan, R., LAU, K. K. and Da, N. P. (1989). Antiperoxidative action of flavonoids and related products in ground pork. *Proc. 3rd Int. Symposium on Flavonoids in Biol. Med.* 56.

Ramos, S. (2008). Cancer chemoprevention and chemotherapy: Dietary polyphenols and signalling pathways. *Mol. Nutr. Food Res.* **52**, 507-526.

Rao, A. L., Bharani, M. and Pallavi, V. (2006). Role of antioxidants and free radicals in health and disease. *Adv. Pharmacol. Toxicol.* **7**, 29-38.

Rhodes, N. (1996). Physiologically active compounds in plant foods; an overview. *Proc. Nutri. Society*. **55**, 371-384.

Rice-Evans, C. A. and Diplock, A. T. (1993). Current status of antioxidant therapy. *Free Radic Biol. Med.* **15**, 77-96.

Rock, C. L., Jacob, R. A. and Bowen, P. E. (1996). Update on biological characteristics of the antioxidant micronutrients- Vitamin C, Vitamin E and the carotenoids. *J. Am. Diet Assoc.*, 693–702.

Ross, J. and Kasum, C. (2002). Dietary flavonoids: bioavailability, metabolic effects and safety. *Am. Rev. Nutr.* (22), 19-34.

Roussel, A. M., Hininger, I., Benaraba, R., Ziegenfuss, T. N. and Anderson, R. A. (2009). Antioxidant effects of a cinnamon extract in people with impaired fasting glucose that are overweight or obese. *J. Am. Coll. Nutri*. **28** (1), 16-21.

Rowland, I. (1999). Optimal nutrition: fibre and phytochemicals. *Proc. Nutri. Society*. **58**, 415-419.

Satyal, P., Dosokyb, N. S., Kincerb, B. L. and Setzera, W. N. (2012). Chemical compositions and biological activities of *Amomum subulatum* essential oils from Nepal. *Nat. Prod. Commun.* **7** (9), 1233-1236.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2001). Phytochemistry of medicinal plants. *J. Pharmaco. and Phytochem.* **1** (6), 168.

Scalbert, A., Manach, C., Morand, C., Remesy, C. and Jimenez, L. (2005). Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutri.* **45**, 287-306.

Shahidi, F. (1992). Antioxidants in food and food antioxidants. *Nahrung/Food*. **44** (3), 158-163.

Sharma, E., Sharma, R., Singh, K. K. and Sharma, G. (2000). A boon for mountain populations: large cardamom farming in the Sikkim Himalaya. *Mountain Research and Development*. **20** (2), 108-111.

Shelef, A. (1984). Antimicrobial effect of spices. *J. Food Safety*. **6** (1), 29-44.

Singh, T. P. and Singh, O. M. (2011). Phytochemical and pharmacological profiles of *Zanthoxylum aramatam* DC- an overview. *Ind. J. Natural products and Resources*. **2** (3), 275-285.

Skowyra, M. (2014). Antioxidant properties of extracts from selected plant materials (*Caesalpinia spinosa*, *Perilla frutescens*, *Artemisia annua* and *Viola wittrockiana*) in-vitro and in model food systems. Ph.D Dissertation. Univ. Barcelona,

Sofowara, A. (1993). "Medicinal plants and Traditional Medicine in Africa". Spectrum Books Ltd. Ibadan. Nigeria.

Su, L., Yin, J., Charles, D., Zhou , K., Moore, J. and Yu, L. (2005). Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chem.*, 990–997.

Subash Babu, P., Prabuseenivasan, S. and Ignacimuthu, S. (2007). Cinnamaldehyde-a potential antidiabetic agent. *Phytomed.* **14** (1), 15-22.

Sunder, S. (2016). Spices as Flavouring Substances. *In:* "Spices Handbook" (3rd ed.). (S. Shirata, Ed.). p. 34. Banglore. Foretell Business Solutions Pvt Ltd.

Suppapitiporn, S., Kanpaksi, N. and Suppapitiporn, S. (2006). The effect of *Cinnamon cassia* powder in type 2 diabetes mellitus. *J. Med. Assoc. Thai.* **89**, 200-205.

Swadhini, S. P. (2011). Phytochemical screening and antimicrobial activity of five medicinal plants against *Myrothecium* spp. *Int. J. Pharma. and Bio. Sci.* **2** (1), 272-279.

Thomas, V. P., Sabu, M. and Gupta, U. (2009). Taxonomic studies on cultivars of *Amomum subulatum*. *Rheedea*. **19** (1), 25-36.

Trease, G. E. and Evans, W. C. (1989). "Pharmacognsy" (11 ed.). Macmilliam Publishers.

Ugwuona, F. U. (2014). Phytochemical composition, antioxidant and antimicrobial properties of four nigerian spices. Ph.D. thesis. Univ. Nigeria, Nigeria.

Vavaiya, R. B., Patel, A. and Manek, R. (2012). Anti-diabetic activity of *Amomum Subulatum* Roxb. fruit constituents *Int. J. Pharma. Innovations*. **2** (5).

Vaya, J. and Aviram, M. (2001). Nutritional antioxidants mechanism of action, analysis of activities and medicinal applications. *Curr. Med. Chem.* **1** (1), 99-117.

Vignoli, J. A., Bassoli, D. G. and Benassi, M. T. (2011). Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chem.* **124**, 863-868.

Wardini, T. H. and Thomas, A. (2016). *Elettaria cardamomum* (PROSEA). *Plant use*.

Waterhouse, A. (2002). Determination of total phenolics. *Curr. Prot. in Food Anal. Chem.*

Yashin, A., Yashin, Y., Xia, X. and Nemzer, B. (2017). Antioxidant activity of spices and their impact on human health: a review. *Antioxidants*. **6** (70).

Zahin, M., Aqil, F. and Ahmad, I. (2009). The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. *Int. J. Pharm. Pharm. Sci.* . **1**, 1-8.

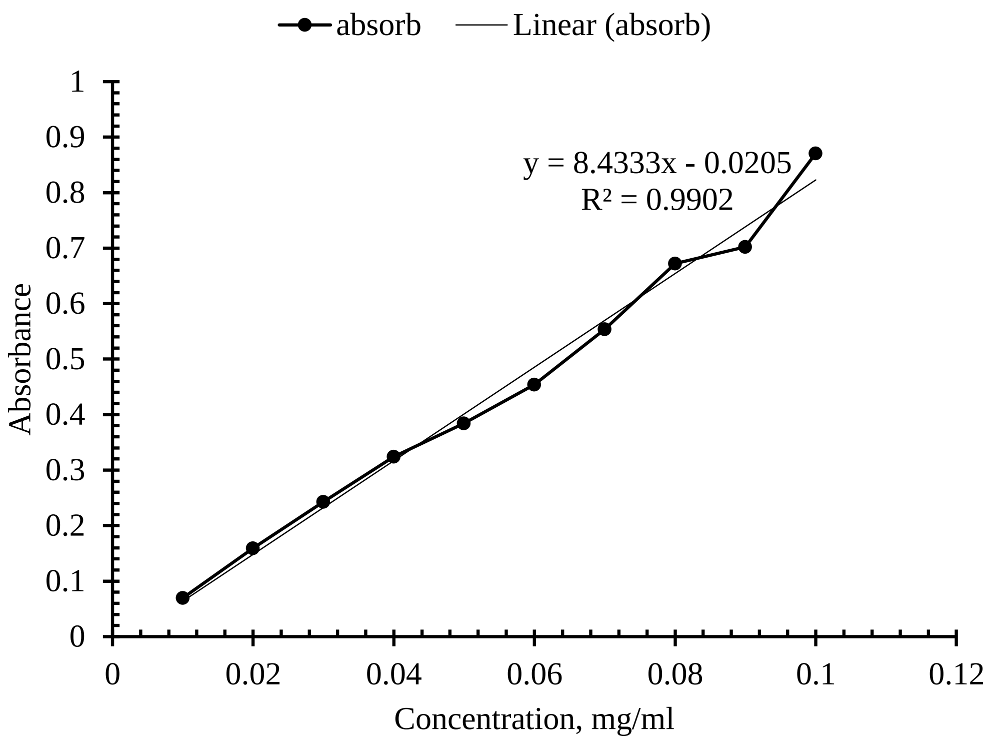
Zheng, J., Y, Z., Li, Y., Xu, D. and Li, H. (2016). Spices for prevention and treatment of cancers. *Nutri*.

Appendices

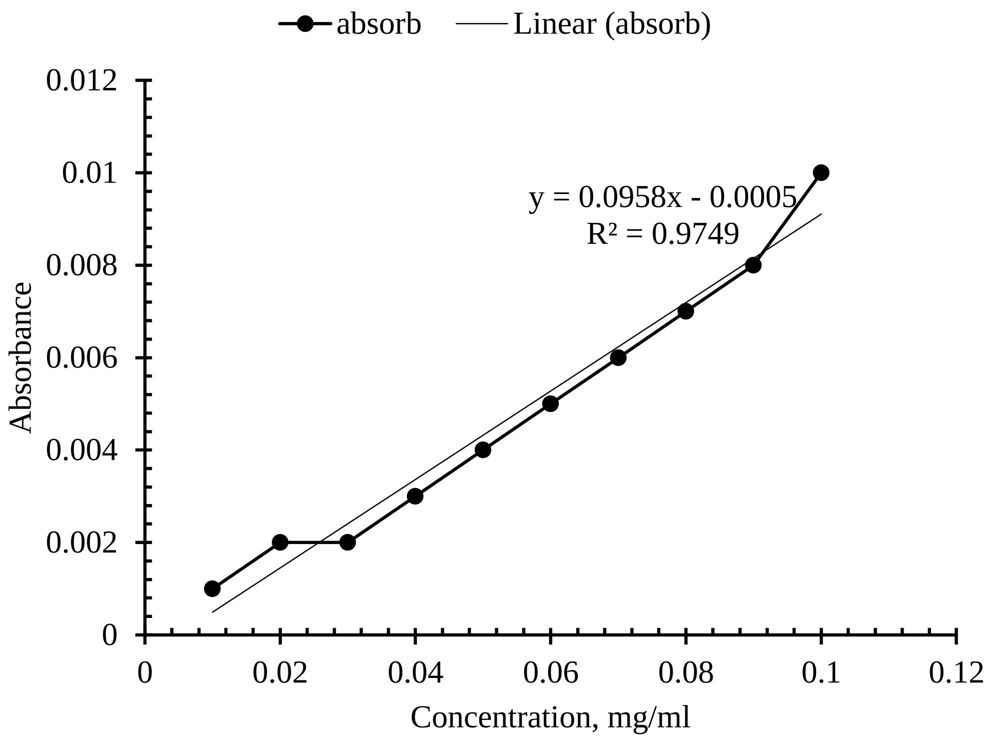
Appendix A

Calibration Curves

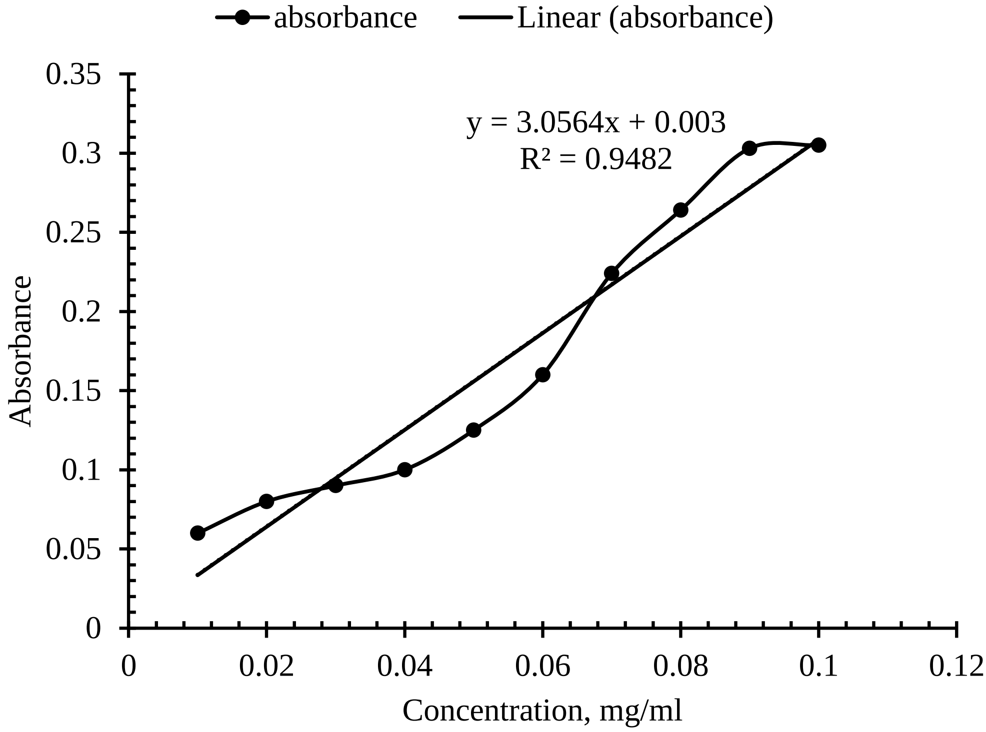
1. Calibration curve of gallic acid for Total Phenol Content



2. Calibration curves of quercetin



3. Calibration curves of gallic acid for tannin



Appendix B

ANOVA tables for spices

Table B.1.1 One way ANOVA (no blocking) for antioxidant

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	48.8991	12.2248	87.22	<.001
Residual	10	1.4016	0.1402		
Total	14	50.3007			

Table B.1.2 One way ANOVA (no blocking) for flavonoid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	177764.11	44441.03	643.25	<.001
Residual	10	690.88	69.09		
Total	14	178454.99			

Table B.1.3 One way ANOVA (no blocking) for phenol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	4	16727.12	4181.78	374.26	<.001
Residual	10	111.73	11.17		
Total	14	16838.86			

Table B.1.4 One way ANOVA (no blocking) for tannin

Source of variation	d.f.	s.s.	m.s.	F pr.
Sample	4	48.8991	12.2248	<.001
Residual	10	1.4016	0.1402	
Total	14	50.3007		

Anova table for Spiced mixture

Table B.2.1 One way ANOVA (no blocking) for phenol

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
M	4	4108.891	1027.223	2946.92	<.001
Residual	10	3.4857	0.3486		
Total	14	4112.377			

Table B.2.2 One way ANOVA (no blocking) for flavonoid

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
mixture	4	44347.08	11086.77	33100.1	<.001
Residual	10	3.3495	0.3349		
Total	14	44350.42			

Table B.2.3 One way ANOVA (no blocking) for tannin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
M	4	13.59491	3.39873	57.43	<.001
Residual	10	0.5918	0.05918		
Total	14	14.18671			

Table B.2.4 One way ANOVA (no blocking) for antioxidant activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
M	4	180.0381	45.00952	865.44	<.001
Residual	10	0.52007	0.05201		
Total	14	180.5581			

Color plates

P1 Analysis of sample

P2 Measuring absorbance in Spectrophotometer